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Antileishmanial Resistance – Reasons and Remedies

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Introduction

Visceral leishmaniasis (VL) or Kala-azar is the most severe form of leishmaniasis and is uniformly fatal, if untreated. An estimated 500,000 new cases occur per year, 90% of which occur in the endemic areas of India, Bangladesh, Sudan Nepal and Brazil.[1] More than 100,000 cases of VL occur in India alone every year and the state of Bihar accounts for more than 90% of these.[2] Over the years new therapies have developed for VL e.g. liposomal amphotericin B, oral miltefosine, and paramomycin. Although a number of drugs have now become available for the treatment of leishmaniasis; each have limitation of either toxicity, long course of treatment, need for hospitalization and close monitoring or parenteral administration (except miltefosine). At the same time as these new therapies are becoming available the standard pentavalent antimonials (Sb^v) are being threatened by development of resistance. It is therefore imperative to look into the reasons behind the development of resistance and prevent future resistance.

History of Antimony resistance

Ever since the discovery of pentavalent antimonials about 60 years ago as a therapeutic agent for VL, they have remained the first line of treatment all over the world. The first indication of drug resistance came from North Bihar in early 80's of about 30% patients not responding to the prevailing regimen of Sb^v.[3] Then a two 10-day courses with a 10-day interval therapy with sodium antimony gluconate was recommended by an expert committee.With this regimen, only 1% patients were refractory to Sb^v therapy in hyperendemic areas. However, in1984, it was seen that with 20 mg/kg (maximum 600 mg) for 20 days, 86% of patients were cured and cure rate with 10mg/kg was quite low.[4] In the same year, the WHO expert committee recommended that pentavalent antimony be used in doses of 20 mg/kg up to a maximum of 850 mg for 20 days, and a repetition of similar regimen for 20 days in cases of treatment failures. The efficacy of the 20 day regimen continued to fall over the years and duration of treatment was increased. In 1997, 156 patients were randomized in three arms for treatment either with (a) Sb^v alone for 30 days, or (b) Sb^v plus interferon-γ (IFN-γ) for 15 days or (c) Sb^v plus IFN-γ for 30 days. 36% patients i.e. only 1/3rd were cured with Sb^v alone, and addition of IFN-γ could improve the cure rate to 42 and 49% in groups b and c, respectively.[5] During the same period only 2% of patients from neighboring UP failed Sb^v treatment.[6] These studies confirmed that a high level of antimony resistance existed in Bihar whereas it was still effective in surrounding areas and the end result was that Sb^v no longer remained the drug of choice in North Bihar.

Reasons for drug resistance – Lessons to be learned

Sb^v was freely available in India, both qualified medical practitioners and quacks used the drug and this unrestricted availability of the drug led to rampant misuse. Almost 73% patients consulted unqualified practitioners first, most of them did not use the drug appropriately.[7] It was a common practice to start with a small dose and gradually build up to the full dose over a week; it was also advocated to have drug free periods to minimize the toxicity, especially renal toxicity and physicians split the daily dose in two injections. These practices resulted in build-up of subtherapeutic blood levels and increased tolerance of parasites to Sb^v.

In a survey of 312 patients who had received one or more courses of antimony but failed to recover. Only 26% were treated according to the WHO guidelines, 42% did not take the drug regularly and 36% stopped the drug on their own initiative.[8] Almost half of the patients, receiving pentamidine as a second-line drug, had not received adequate antimony treatment before being labeled as refractory to Sb^v. These facts indicated large-scale misuse of antileishmanial drugs in Bihar, contributing to development of drug resistance.

There were several manufacturers of Sb^v in India, and quality of products were inconsistent, resulting in occasional batches being substandard and toxic, this added to the problems associated with Sb^v therapy causing serious toxicity and deaths related to the drug.

There had been speculations (i) whether Indian *Leshmania donovani* had become truly refractory to Sb^v or (ii) resistance occurred because of the inadequate doses being used in Bihar, or (iii) whether there were unknown host factors which determined the response to treatment. In a study to determine whether acquired drug resistance was present in Bihar, *L. donovani* isolates were taken from responders and nonresponders. In vitro amastigote-macrophage assay showed that isolates from patients who did respond to sodium stibogluconate treatment were threefold more sensitive, with 50% effective doses (ED_{50}s) around 2.5 µg Sb/ml compared to isolates from patients who did not respond (ED_{50}s around 7.5 µg Sb/ml).[9] The significant difference in amastigote sensitivity supported the concept of acquired resistance in Bihar. However, further increase in dose could not be recommended as serious and fatal toxicity associated with the current regimen were at the limits of acceptability, and increasing the dose of Sb^v any further would seriously jeopardise the safety of the patients.

Another reason for the growing resistance to Sb^v in India while it still remained sensitive all over the world is due to the fact that leishmaniasis usually has zoonotic transmission except in the Indian subcontinent and East Africa where the transmission is anthroponotic. Once there is emergence of Sb^v refractory parasites in the anthroponotic cycle, they circulate in the community efficiently as Sb^v sensitive parasites get eliminated by the drug, and the proportion of patients with Sb^v refractory parasites rises.

Are other Antileishmanials at higher risk?

The main reasons for antimony resistance was subtherapeutic doses, incomplete duration of treatment and substandard drugs perpetuated by an anthroponotic cycle.Therefore similar fate awaits all the other drugs, if proper precautions are not taken.

Pentamidine is another antileishmanial which suffered the same fate as Sb^v.Pentamidine was the first drug to be used in patients refractory to Sb^v and cured 99% of these patients initially.[10] In the next two decades; however, its efficacy dwindled to approximately 70% of patients.[11] Its use was ultimately abandoned due to its decreased efficacy and serious toxicities.

Amphotericin B is now being used as a first line therapy in areas with Sb^v resistance.It has excellent cure rates ($> 97\%$) at doses of 0.75–1.00 mg/kg for

15 infusions on alternate days.[12] It has been used extensively in Bihar with uniformly good results. The high cost, need for prolonged hospitalization, intravenous administration and occasional severe adverse reaction like hypokalemia, thrombocytopenia, myocarditis and death are some of the drawbacks of this excellent drug. Special amphotericin B treatment centers with trained personnel and free supply of drugs need to establish in these areas to promote its proper use.

Lipid-associated amphotericin (L-AB) preparations (AmBisome and Abelcet) are as effective as conventional amphotericin B, and have negligible adverse reactions.[13] It is possible to administer high doses of L-AB over a short period with high cure rates; however, their high cost makes these compounds unaffordable in the endemic areas.

Miltefosine an alkyl phospholipid is the first oral agent approved for the treatment of leishmaniasis. At the recommended doses (100 mg daily for patients weighing ≥ 25 kg and 50 mg daily for those weighing < 25 kg for 4 weeks) cure rates were > 95%.[14] As it is effective in Sb^v resistant cases it can be used as a first line drug in areas with >10% Sb^v unresponsiveness. Being an oral agent it offers an advantage of improved compliance, self administration and reduced costs of admission. Miltefosine with its excellent efficacy, oral administration and good tolerance can be an important tool in containing the epidemic. However the easy availability of the drug over the counter, high cost of the total therapy could lead to the intake of inadequate dose of the drug for shorter duration. As the drug has a long half-life (approximately 150 h), this could lead to subtherapeutic drug level for a prolonged period and ultimately widespread resistance.

Paromomycin, an aminoglycoside antibiotic was approved by the Indian government in August 2006 for the treatment of patients with visceral leishmaniasis. It is administered intramuscularly at a dose of 11 mg per kilogram daily for 21 days and has shown overall cure rate of 95%. The cure rate among those whose disease had not responded to previous treatment with sodium stibogluconate or miltefosine or who had had a relapse was high (98%).[15] Since paromomycin has not yet been used extensively, resistance is not a problem in the field, nevertheless, monitoring of resistance needs to be done.

Policies to prevent the appearance and spread of antileishmanial resistance

Free distribution of drugs

The high cost of the antileishmanial drugs coupled with easy, over the counter availability often leads to under dosing and incomplete treatment. This has been the major factor for antimony resistance and could lead to resistance to other drugs as well especially the novel oral agent miltefosine. Considering that majority of the population cannot afford to purchase and complete a full course of treatment it is recommended that antileishmanials should be made available free of cost to be distributed through public and/ or private health care providers like Antitubercular and Antiretroviral drugs.

Directly observed Therapy

The directly observed treatment strategy for tuberculosis has been a big success and a parallel system could be evolved for leishmaniasis especially with oral drugs. This will lead to better compliance, completion of the treatment course and ultimately prevent resistance.

Combination therapy

Growing resistance of the parasite to antileishmanial drugs suggests that the currently used monotherapy needs to be reviewed. Multidrug combination therapy has been used successfully in tuberculosis, leprosy and malaria. The rationale behind combination therapy are (i) increased activity through use of compounds with synergistic or additive activity, (ii) preventing the emergence of drug resistance, (iii) lower dose requirement thereby reducing chances of toxic side effects and cost, and (iv) increased spectrum of activity. Studies to identify such combination in leishmaniasis need

to be undertaken, as it will shorten the duration of therapy, improve compliance and decrease the development of resistance. In India amphotericin B and its lipid formulations, miltefosine and paromomycin are some of the drugs which can be combined.

Monitoring drug resistance

Ideally, parasite resistance should be monitored, rather than patient relapse rates. It will also permit the identification of key intracellular targets and parasite defense mechanisms, which can then be exploited to rationally develop analogs of existing drugs that would not get affected by the most common defenses. Analysis of genetic markers that determine high antileishmanial resistance, performed systematically for every parasite isolate that shows low antileishmanial sensitivity would facilitate the tracking of the level of resistance in affected populations. At present no molecular markers of resistance are available for the currently used antileishmanial drugs and the only reliable method for monitoring resistance of isolates is the technically demanding in vitro amastigote-macrophage model. Development of drug resistance markers and tools easy to use in the field should be encouraged.

New targets, new drugs

There are few better ways to avoid drug resistance than to have an adequate armory of drugs with different targets and no cross-resistance.

Training and Health education programs

One of the major reasons for antimony resistance was lack of awareness among the affected population and health care providers, about the need for effective treatment and control of kala-azar. There is a considerable need and scope for orientation programs to educate those at risk, doctors and the government agencies responsible for controlling and preventing kala-azar in India.

Management of HIV/VL co-infection

Another potential source for the emergence of drug resistance are the HIV/VL coinfected patients. These patients have high parasite burden, a weak immune response, respond poorly to treatment and have a high relapse rate.Therefore they are the ideal candidates to harbor drug resistant parasites. With the growing burden of HIV in India, HIV/VL coinfection could become a major problem. Experience from Southern Europe shows that initial response to Sb^{v} and conventional amphotericin B is low (~40-65%) in severely immunocompromised persons and severe adverse events are frequent. The best results are observed with AmBisome.It has also been observed that initiation of HAART dramatically decreases the incidence of VL coinfection. Therefore, HAART in combination with antileishmanials should be advocated strictly in these patients.

Conclusion

Few drugs are available for treating *Leishmania* infections and the emergence of drug resistance is further complicating the control of leishmaniasis. A better understanding of resistance mechanisms and mechanism of action of drugs may point the way to more rational uses of drugs. Combination chemotherapy is rapidly emerging as the norm for treating several parasitic infections and is strongly advocated for kala-azar. Directly observed therapy given free, in treatment centers manned by trained personnel will go a long way in controlling the disease as well as drug resistance.

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