Available online at www.sciencedirect.com
SCIENCE DIRECT®
Acta Tropica 94 (2005) 41–47
ACTA TROPICA
www.elsevier.com/locate/actatropica

Efficacy of picroliv in combination with miltefosine, an orally effective antileishmanial drug against experimental visceral leishmaniasis

Suman Gupta [a,*], Ramesh [a], S.C. Sharma [b], V.M.L. Srivastava [a]

[a] *Divisions of Parasitology, Central Drug Research Institute, Post Box 173, Lucknow 226001, India*
[b] *Division of Medicinal and Process Chemistry, Central Drug Research Institute, Post Box 173, Lucknow 226001, India*

Received 11 May 2004; accepted 18 November 2004
Available online 8 March 2005

Abstract

Visceral leishmaniasis (VL) or kala-azar continues to persist as one of the major public health problems in many tropical countries. However, no effective treatment for radical cure of the disease is yet available. Miltefosine, an alkyl phospholipid compound, is the first orally effective drug, which has shown 98% cure rate of VL patients during phase III clinical trial conducted in India. Since this drug requires long course of treatment and has long half-life, there are fairly good chances of emergence of resistance. Furthermore, this drug has produced severe side effects in some of the cases. We therefore examined the possibility of minimizing these effects by applying miltefosine in lower doses in combination with picroliv, an immunomodualtor against *Leishmania donovani* in hamsters (*Mesocricetus auratus*).

The picroliv per se showed no antileishmaial potential. However, when given with suboptimal dose of miltefosine, it enhanced efficacy of the latter from 45 to 86% on day 7 post treatment and from 32 to 64% on day 28 post treatment. Interestingly, the effectivity of this combination was as good as that of the curative dose of miltefosine alone. Thus, this combination appears to offer a fruitful strategy for treatment of VL.

Keywords: Visceral leishmaniasis; *Leishmania donovani*; Picroliv; Miltefosine; Hamster; Combination therapy

1. Introduction

Visceral leishmaniasis (VL) or kala-azar, a disease affecting 61 out of the 88 countries worldwide is caused by kinetoplastid protozoan parasite belonging to the *Leishmania donovani* complex (WHO, 1996). According to a WHO estimate there are at least 3–5 million clinical cases among the 12 million infected individuals from a total population of about 350 million living in endemic areas (WHO, 1998). Approximately, 1.5 million new cases are reported each year, of which nearly 50,000 suffer from the disease.

* Corresponding author. Tel.: +91 522 2212411/2212418x4428; fax: +91 522 2223405/2223938.
E-mail address: gupta_suman@yahoo.com (S. Gupta).

0001-706X/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2004.11.009

In India, Bihar is the highly endemic state but the disease has spread to newer areas also (Jha et al., 1998). Epidemics have been seen in eastern Uttar Pradesh and West Bengal (Gupta et al., 1997; WHO, 1999; Kumar et al., 1999) but sporadic cases have also been reported from other parts of the country, where kala-azar was never seen before (Thakur, 1984; Kumar et al., 1999). Antimonials, the mainstay of treatment have no more practical use because of resistance (Jha et al., 1998) and the use of traditional second-line drugs (pentamidine and amphotericin-B) is limited due to toxicity and inadequate supply. Alternatives are a few and the most effective compounds today like lipid formulations of amphotericin-B, are simply unaffordable due to high cost. No treatment has proven effective in achieving radical cure of VL when associated with HIV infection (Berenguer et al., 1989; Alvar et al., 1996; WHO, 1999). Alkyl phospholipid compound, miltefosine is the first effective oral compound for VL and is under Phase IV clinical trial (Sundar et al., 1999; WHO, 2002).

Since immunodeficiency is associated with this disease, combination therapy employing an immunostimulant with an antileishmanial drug appears to be a fruitful and promising strategy (Murray et al., 1988; Badaro et al., 1994). We therefore examined this approach in *L. donovani* infected hamsters using combination of miltefosine and picroliv, an immunostimulant (Puri et al., 1992; Chander et al., 1994; Rastogi et al., 1996).

2. Materials and methods

2.1. Parasite

The WHO reference strain of *L. donovani* (MHOM/IN/80/Dd8) obtained from Imperial College, London (UK) in 1979, has been maintained since then in this laboratory as promastigotes in vitro in NNN medium, and as amastigotes in golden hamster.

2.2. Animal

Golden hamsters (*Mesocricetus auratus*) of both sexes weighing 45–50 g and bred in the National Animal Laboratory Center housed in CDRI, Lucknow, served as the experimental host. They were maintained in a temperature regulated room and were provided with standard rodent pellet diet (Nav Maharashtra Chakan oil Mills, Pune, Maharashtra, India) and water ad libitum.

2.3. Compound

Miltefosine (hexadecyl phosphocholine) was received from Sigma Chemical Co., USA, while picroliv was isolated from *Picrorhiza kurooa* (family: Scrafulariaceae) a perennial herb growing in the Himalayan region at the height in between 9000 and 13,000 ft. Its root and rhizomes have long been used in the ayurveda and traditional system of medicine in India for the treatment of fever, jaundice and various types of liver ailments. The method has been described in detail by Dwivedi et al. (1991).

2.4. In vivo evaluation of picroliv in combination with miltefosine in patent infection

For in vivo evaluation of picroliv, miltefosine and their combination, the method of Beveridge (1963) as modified by Bhatnagar et al. (1989) was employed. Briefly, intracardially infected hamsters were biopsied on days 17–18 p.i. and animals showing +1 infection (5–15 amastigote per 100 cell nuclei) were selected. Twenty–thirty hamsters were randomized into five groups on the basis of their parasitic burden. Treatment with miltefosine/picroliv by p.o. route was initiated after 2 days of biopsy. Miltefosine was given for 5 consecutive days while picroliv was continued upto 33 days. Hamsters of Group A were given picroliv at 10 mg/kg dose, while those of Group C were treated with suboptimal dose of miltelfosine (25 mg/kg) along with picroliv. The animals of Group B received miltefosine at suboptimal dose of 25 mg/kg. The animals of Group D received miltefosine at curative dose (50 mg/kg) and those of Group E receiving SHAM treatment of distilled water served as untreated controls. The experiment was repeated twice for statistical evaluation.

In the first two experiments, post treatment biopsy was done on day 7 as well as on day 28 of the last dose of miltefosine while in the third experiment it was done only once on day 28. Amastigote count was assessed by Geimsa staining. Percent inhibition in amastigote multiplication was calculated using the