Combination Therapy with Indolylquinoline Derivative and Sodium Antimony Gluconate Cures Established Visceral Leishmaniasis in Hamsters

Chiranjib Pal,1† Mousumi Raha, 2 Anirban Basu,1‡ Keshab Chandra Roy,1 Anasuya Gupta,3 Monidipa Ghosh,1 Niranjan Prasad Sahu,2 Sukdeb Banerjee,2 Nirup Bikash Mandal,2 and Santu Bandyopadhyay1*  

Immunology Division1 and Steroid and Terpenoid Chemistry Division, 2 Indian Institute of Chemical Biology, Jadavpur, Kolkata-700 032, and Department of Zoology, Calcutta University, Kolkata-700 019, 3 India

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2-(2′-Dichloroacetamidobenzyl)-3-(3′-indolylquinoline), designated indolylquinoline derivative A, reduced the splenic and the liver parasite burdens by >93.0% in Leishmania donovani-infected hamsters, whereas sodium antimony gluconate (SAG) reduced the burdens approximately 80.0%. Complete clearance of parasitemia from the livers and spleens was noticed when infected animals received indolylquinoline derivative A plus SAG, suggesting that indolylquinoline derivative A has potential as a new agent for sole or conjunctive therapy for leishmaniasis.

The visceral form of leishmaniasis, commonly known as kala azar, is caused by the parasite Leishmania donovani. Approximately 350 million people in 8 countries are estimated to be threatened by the disease (14). The World Health Organization estimated in 2000 that there were 12 million cases of all forms of leishmaniasis worldwide, with over 500,000 new cases of visceral disease occurring each year (14). Despite the tremendous progress that has been made in understanding the biochemistry and molecular biology of Leishmania species, chemotherapeutic treatment for visceral leishmaniasis has seen limited progress in recent years. The toxic pentavalent antimonials remain the mainstay of treatment for leishmaniasis. The second-line drugs, pentamidine and amphotericin B, although used clinically, have serious toxic side effects (8). To make the situation even worse, some parasite strains have developed resistance to the classical antimonial drugs (4, 13). However, recent reports have suggested that amphotericin B-lipid complexes have efficacy for the treatment of antimony-unresponsive visceral leishmaniasis (10, 12). Miltefosine, an orally active phosphocholine analogue, also appeared to be effective in the treatment of visceral leishmaniasis (3, 11). Despite these advances, improved drug therapy for visceral leishmaniasis still remains desirable.

We previously reported that indolylquinoline derivatives including 2-(2′-dichloroacetamidobenzyl)-3-(3′-indolylquinoline), designated indolylquinoline derivative A, were cytotoxic to L. donovani promastigotes and amastigotes in vitro and were effective in the treatment of murine visceral leishmaniasis (2). In the present study, we investigated the efficacy of combination therapy with indolylquinoline derivative A and sodium antimony gluconate (SAG) against visceral leishmaniasis in hamsters.

Golden hamsters were infected with a pathogenic strain of L. donovani (strain AG83) as described previously (2). Briefly, male golden hamsters (age, 4 to 6 weeks) were injected by the cardiac route with freshly prepared L. donovani amastigotes (106 amastigotes/hamster). Therapy with indolylquinoline derivative A or SAG, or both, started at 4 weeks postinfection. Indolylquinoline derivative A (Fig. 1) was synthesized from indole by the Friedel-Crafts acylation reaction as described previously (5). Briefly, the reaction was carried out with indole (3 to 4 mol), acylchloride (1 mol), and anhydrous aluminium chloride (1.5 to 2 mol). The reaction mixture was kept at 25°C for 1 h, warmed to 85°C for 4 h, and then kept overnight at room temperature. The reaction mixture was treated with an ice-HCl (1:1) mixture, neutralized with sodium bicarbonate solution, and extracted with chloroform. The extract was evaporated under reduced pressure. The concentrated gummy mass was adsorbed with silica gel and subjected to column chromatography. A stock solution of indolylquinoline derivative A was prepared in dimethyl sulfoxide (DMSO) at 10 mg/ml, and further dilutions were made in phosphate-buffered saline.

Infected hamsters received the indicated compounds once a week for 4 weeks through the intramuscular route and were killed at 9 weeks postinfection. The splenic and the liver parasite burdens were determined from impression smears after Giemsa staining, and the results are expressed as the total parasite load per organ, as described previously (9), using the following formula: organ weight (in milligrams) × number of amastigotes per cell nucleus × (2 × 107). For statistical analyses, Student’s t test was used with the program Tadpole III (written by T. H. Caradoc Davies, Wakari Hospital, Dunedin, New Zealand [published and distributed by Biosoft, Cambridge, United Kingdom]). Treatment of infected hamsters with a low dose of indolylquinoline derivative A (10 mg/kg of body weight) had no appreciable inhibitory effect on the level
of parasitemia in the spleen; however, the liver parasite burdens were significantly reduced (59.7% inhibition; \( P < 0.05 \)) (Fig. 2). By increasing the dose of indolylquinoline derivative A to 40 mg/kg of body weight, dramatic inhibition of parasitemia in both organs, i.e., the spleen and the liver, was achieved. The reduction of the parasite burden was >93% in both organs (\( P < 0.007 \) for each comparison). No more increase in antileishmanial activity was achieved by further increasing the dose of indolylquinoline derivative A to 50 mg/kg of body weight. SAG alone at a dose of 20 mg/kg of body weight reduced the splenic and liver parasite burdens almost equally (approximately 80%; \( P < 0.01 \) for each comparison). Interestingly, complete clearance of parasitemia from the spleen and the liver was noticed when infected animals received combination therapy with indolylquinoline derivative A (40 mg/kg of body weight) and SAG (20 mg/kg of body weight). To further evaluate whether these organs had living parasites, homogenates of these organs were cultured for 2 weeks after serial dilution as described previously (7). No transformed promastigotes were detected in this group of animals, although promastigotes were readily detectable from animals receiving DMSO, SAG, or indolylquinoline derivative A.

To check the liver function, the serum alkaline phosphatase (ALP), serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT) levels of hamsters undergoing experimental visceral leishmaniasis and receiving therapy were assayed by using the kits from Dr. Reddy’s Laboratories, Hyderabad, India, according to the

![FIG. 1. Structure of indolylquinoline derivative A [2-(2'dichloroacetamidobenzyl)-3'(indolylquinoline)].](image1)

![FIG. 2. Combination therapy for established visceral leishmaniasis in hamsters with indolylquinoline derivative A plus SAG. Animals received the indicated treatment at 4 weeks postinfection, and the parasite burden was determined at 9 weeks postinfection. Data represent the means ± standard deviations for three to five animals per group. *, \( P < 0.05 \); **, \( P < 0.01 \); ***, \( P < 0.007 \).

Graph showing parasite burden in spleen and liver for different treatments.
FIG. 3. Specific enzyme levels in sera of hamsters undergoing experimental visceral leishmaniasis. Enzyme assays were performed with kits from Dr. Reddy's Laboratories according to the manufacturer's instructions. Animals received the indicated treatment at 4 weeks postinfection and were killed at 9 weeks postinfection for collection of sera. Data represent means ± standard deviations for three to five animals per group. *, P < 0.01; **, P < 0.005; ***, P < 0.002; ****, P < 0.001. Normal, uninfected control; DMSO, infected control; KAU, KA units; Indo A, indolylquinoline derivative A; B.W., body weight.

manufacturer's instructions. ALP activity was expressed as KA units per deciliter, where 1 KA unit/dl is equal to 7.1 U/liter (7). SGPT and SGOT activities were expressed as units per milliliter (6). L. donovani infection had differential effects on serum ALP, SGOT, and SGPT levels (Fig. 3). The serum ALP level was significantly higher (P < 0.002) in L. donovani-infected hamsters than in uninfected controls. On the other hand, L. donovani infection significantly reduced the SGPT level (P < 0.005), and the level of SGOT was reduced only marginally. The level of ALP decreased significantly in infected hamsters upon receipt of indolylquinoline derivative A or SAG, or both (P < 0.01 for each comparison compared to the results for infected hamsters receiving DMSO alone). However, the level was still higher than normal in each hamster. In contrast, the levels of both SGOT and SGPT in infected hamsters increased, approaching normal values upon receipt of these treatments, although the increase was significant only in the case of SGPT (P < 0.001 for each comparison). These results suggest that indolylquinoline derivative A is nontoxic to the liver up to a dose of 50 mg/kg of body weight.

We have previously reported that indolylquinoline derivatives have antileishmanial properties (2). One of the derivatives, designated indolylquinoline derivative A, was also effective in reducing the splenic parasite burden in BALB/c mice. However, complete clearance of parasitemia was not achieved with this compound. Here we report that combination therapy with indolylquinoline derivative A and SAG, a classical antileishmanial drug, resulted in complete clearance of parasitemia from the livers and spleens of L. donovani-infected hamsters. The antileishmanial property of pentavalent antimony has reportedly been enhanced by combining the pentavalent antimony with gamma interferon (IFN-γ) (12, 13) or an IFN-γ inducer (1). Residual parasites were still detectable in most of the patients or animals receiving the combination therapy. In contrast, the present report suggests that combination therapy with indolylquinoline derivative A and SAG left no residual living parasites in the livers and spleens of infected animals. Taken together, our data suggest that indolylquinoline derivative A may be a new agent for sole or conjunctive therapy of leishmaniasis with minimal toxicity.

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