Treatment options for visceral leishmaniasis: a systematic review of clinical studies done in India, 1980–2004

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The state of Bihar in India carries the largest share of the world's burden of antimony-resistant visceral leishmaniasis. We analysed clinical studies done in Bihar with different treatments between 1980 and 2004. Overall, 53 studies were included (all but one published), of which 15 were comparative (randomised, quasi-randomised, or non-randomised), 23 dose-finding, and 15 non-comparative. Data from comparative studies were pooled when appropriate for meta-analysis. Overall, these studies enrolled 7263 patients in 123 treatment arms. Adequacy of methods used to do the studies and report on them varied. Unresponsiveness to antimony has developed steadily in the past to such an extent that antimony must now be replaced, despite attempts to stop its progression by increasing dose and duration of therapy. The classic second-line treatments are unsuited: pentamidine is toxic and its efficacy has also declined, and amphotericin B deoxycholate is effective but requires hospitalisation for long periods and toxicity is common. Liposomal amphotericin B is very effective and safe but currently unaffordable because of its high price. Miltefosine—the first oral drug for visceral leishmaniasis—is now registered and marketed in India and is effective, but should be used under supervision to prevent misuse. Paromomycin (or aminosidine) is effective and safe, and although not yet available, a regulatory submission is due soon. To preserve the limited armamentarium of drugs to treat visceral leishmaniasis, drugs should not be deployed unprotected; combinations can make drugs last longer, improve treatment, and reduce costs to households and health systems. India, Bangladesh, and Nepal agreed recently to undertake measures towards the elimination of visceral leishmaniasis. The lessons learnt in Bihar could help inform policy decisions both regionally and elsewhere.

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Introduction

Over 90% of the global total of visceral leishmaniasis cases occur in five countries across three continents: north eastern India, Bangladesh, and Nepal in the Indian subcontinent, Sudan in Africa, and north eastern Brazil in South America.[1,2] The situation is particularly grave in the state of Bihar, India, known as the "heartland of kala-azar" (figure 1). Here, the burden of disease has increased steadily in the past;[3] the disease is spreading, and unresponsiveness to antimonials has severely compromised disease control.[4] Today, of all regions, Bihar is facing the most immediate public-health problem, with a lack of suitable treatment options for a growing problem.[5] Current alternative treatments are amphotericin B and its lipid formulations, pentamidine, miltefosine, and paromomycin.[6–8]

In a previous article, we reviewed the overall situation and needs concerning visceral leishmaniasis, and the overall failures to address key priority issues for disease control.[8] Here, we aim to properly document and analyse the efficacy and safety profiles of both in-use and experimental drugs in this part of India to produce reliable summaries in support of policy decisions both in India and elsewhere. Such information is particularly relevant now that India, Bangladesh, and Nepal have decided to undertake measures towards the elimination of the disease as a public-health problem.[9]

Methods

We systematically searched Pubmed and Cochrane databases for articles published from 1980 to 2004 with the keywords "leishmaniasis", "kala-azar", and "treatment". The search was limited to include trials done in Bihar. The search was further refined with the words "antimony", "antimonial", "sodium stibogluconate", "pentamidine", "amphotericin B", "liposomal amphotericin B", "miltefosine", or "paromomycin". The references of the retrieved articles were also reviewed to find additional sources of data. We identified key researchers from the literature search and our own

Figure 1: **The typical environment in Bihar were conditions favour transmission and where kala-azar cases occur**

contacts to find unpublished datasets, including both comparative and non-comparative trials for visceral leishmaniasis treatment done in Bihar. Our own collections of publications were also included. Relevant articles were then reviewed for inclusion and analysed.

We classified studies as comparative (ie, *vs* another drug or a drug combination), dose-finding (ie, comparisons of different dosing regimens of the same drug), or non-comparative (ie, single-arm studies). For comparative and dose-finding studies we assessed adequacy of methods of assignment and concealment of allocation.

We extracted efficacy and safety information from each study. For all, we recalculated exact CIs using Epi-Info. For comparative studies, the odds ratio and 95% CI were calculated using RevMan (RevMan 4.2.6, Cochrane IMS). In this latter case, the number needed-to-treat was also calculated from the risk difference.[10,11]

Results

Overall, 53 studies were included in this review (all but one published), of which 15 were comparative (randomised, quasi-randomised, or non-randomised), 23 dose-finding, and 15 non-comparative. These studies enrolled a total of 7263 patients in 123 study arms. Amphotericin B (deoxycholate and lipid formulations) contributed approximately 50% of all patients and study arms (table 1). The results for each drug are presented below. Table 2 summarises the main characteristics of the regimens studied.

Adequacy of assignment and concealment were difficult to assess because in most cases there was scant information in the papers to that effect. No study was blinded. Safety was reported sporadically and results could not be tabulated. Not all studies provided sufficient information on patient attrition for the assessment of efficacy.

Pentavalent antimony

Urea stibamate was the first antimonial drug, introduced over 70 years ago, later being replaced in the 1950s by sodium stibogluconate, which became the first-line treatment for visceral leishmaniasis. Initially, the drug was used at very low doses (eg, 10 mg/kg per day for 6–10 days).[27] The drug was cheap, and, at that time, effective and well-tolerated. Then, failures started to occur, and a routine of gradually increasing the dose and duration of therapy began in the attempt to catch up with resistance.

In this review we found eight different regimens of antimony alone. The drug has been used with interferon γ, paromomycin (also known as aminosidine), or pentamidine either concomitantly or sequentially.[4,12–17,28–33] We found 13 studies (eight comparative, three dose-finding, and two non-comparative) done between 1980 and 2001, enrolling 1562 patients in a total of 24 antimony study arms (table 3). Adequacy of allocation was difficult to assess, except for studies versus paromomycin. In the three dose-finding studies done in the 1980s, there was a clear correlation (r^2=0·82) between total dose (within the range 200–800 mg/kg) and outcome. At that time, a total dose of 600 mg/kg was 83–86% effective; despite this, 20 mg/kg per day over 30 days was the standard treatment in the 1990s. These data document the progressive erosion of efficacy of antimonials in Bihar, to the extent that since the mid-1990s a regimen of 20 mg/kg per day for 30 days cured only 36–69% of cases.

The results of the eight comparative trials with nine comparator treatments are presented in table 4.[13–17,22–24] No significant benefit was achieved by combining antimony with interferon γ. In all other cases, antimony was significantly less effective than the comparator regimen. The numbers needed-to-treat for a paromomycin 18 mg/kg per day plus antimony combination was two, for amphotericin B three, and for paromomycin 16–20 mg/kg per day alone was four. Thus, one out of two to four patients benefit from another treatment than antimony alone.

The data point to increased toxicity with the increased total dose of antimony.[4,13,17,35] Although direct comparisons of the toxicity profiles of different dosing regimens are not available, with 20 mg/kg per day for 28 days, cardiotoxicity was reported in 8–17% of cases, with 5–7% proving to be fatal.[17]

Amphotericin B and its lipid formulations

We identified 26 trials and 64 study arms treating 3612 patients with amphotericin B in various formulations (ie, 50% of the entire database). Information provided

Drug	Comparative	Dose-finding	Non-comparative	Total studies	Study arms	Total patients	Percentage patients
Antimony	8	3	2	13	24	1562	22
Amphotericin B deoxycholate	7	5	6	18	25	2741	38
AmBisome	2	3	1	6	11	476	7
Amphotericin B lipid complex	1	4	1	6	15	314	4
Amphotericin B fat emulsion	1	..	1	2	2	81	1
Pentamidine	4	..	3	7	11	993	14
Paromomycin	2	2	1	5	15	431	6
Miltefosine	1	6	..	7	20	665	9
Total (studies with multiple arms counted once)	15	23	15	53	123	7263	100

Table 1: **Overview of trials included in the review by drug and type of study**

References	Drug	Route of administration	Selected dosage and schedule	Days in hospital	Drug price (US$/mg)	Average cure rate (%)
Sundar et al[4] Jha et al[12] Sundar et al[13] Thakur et al[14–16] Thakur and Narayan[17]	Antimony*	Intramuscular	20 mg/kg qd for 30 d	30	0·0014	50
Sundar et al[18,20] Thakur et al[19]	Amphotericin B deoxycholate†	Intravenous	1 mg/kg eod for 15 doses over 30 d	30	0·137	97
Sundar et al[21]			2 mg/kg eod for five doses over 10 d in fat emulsion	10	0·137 + 0·0564‡	93
Sundar et al[22,23]	Amphotericin B lipid complex	Intravenous	2 mg/kg eod for five doses over 10 d	10	1·522	90
Sundar et al[24,25]	AmBisome§	Intravenous	5–7·5 mg/kg once	1	4	91–93
Sundar et al[20] Thakur et al[26]			2 mg/kg eod for five doses over 10 d (total dose 10 mg/kg)	10	4	98
Jha et al[12]	Paromomycin‖	Intramuscular	16 mg/kg qd for 21 d	21	0·0044	91
Thakur et al[15]			18 mg/kg + antimony 20 mg/kg	21	0·0044 (paromomycin),	
Thakur et al[14]			qd for 21 d		0·0014 (antimony)	93
Sundar et al[18]	Miltefosine¶	Oral	100 mg qd for 28 d	3	0·052	94

*Based on studies from 1994; †20 mg/kg total dose (over 40 days) is 99% effective, hospital mixture of two separate entities; ‡price of amphotericin B and fat emulsion, respectively; §preferential price to MSF US$0·4/mg (not available in India); ‖results of latest phase III study with 15 mg/kg per day not available; ¶price under negotiation. eod=every other day; qd=once daily

Table 2: **Summary characteristics of regimens considered**

was insufficient to conclude as to the quality of methods of allocation and concealment used, with few exceptions.[18,20,26]

18 studies of amphotericin B deoxycholate enrolled 2741 patients (seven were comparative, five dose-finding, and six non-comparative; table 5). Amphotericin B deoxycholate has been used in different regimens—eg, total dose ranging from 7 mg/kg to 20 mg/kg, and treatment administered on alternate days or daily for up to 43 days at either constant or incremental dosing.[24,26,34,47]

Reference	Years of study	Regimen	Number treated	Number cured	Percentage cured	95% CI	Study type
Thakur et al[30]	1981–82	20 mg/kg per d for 20 d	63	54	85·7	74·6–93·2	Dose-finding
		20 mg/kg per d for >20 d	63	62	98·4	91·5–100	
Thakur et al[29]	1984–87	10 mg/kg per d for 20 d	58	33	56·9	43·2–69·8	Dose-finding
		10 mg/kg per d for 40 d	61	45	73·8	60·9–84·2	
		15 mg/kg per d for 20 d	62	42	67·7	54·7–79·1	
		15 mg/kg per d for 40 d	63	54	85·7	74·6–93·2	
		20 mg/kg per d for 20 d	63	51	81·0	69·1–89·7	
		20 mg/kg per d for 40 d	64	62	96·9	89·1–99·6	
Thakur et al[31]	1988–89	20 mg/kg per d for 20 d	104	74	71·2	61·4–79·6	Dose-finding
		20 mg/kg per d for 30 d	104	86	82·7	74–89·4	
		20 mg/kg per d for 40 d	104	98	94·2	87·8–97·8	
Thakur et al[33]	1991	20 mg/kg per d for 30 d	75	60	80·0	69·1–88·3	RCT *vs* amphotericin B
Mishra et al[32]	1992–93	20 mg/kg per d for 40 d	40	25	62·5	45·8–77·2	RCT *vs* amphotericin B
Sundar et al[28]	1992–93	20 mg/kg per d for 30 d	15	9	60·0	32·3–83·7	RCT antimony *vs* antimony + interferon γ
		20 mg/kg per d for 30 d + interferon γ 100 mg/m²	16	13	81·3	54·3–96	
Thakur et al[16]	1994–96	20 mg/kg per d for 30 d	80	46	57·5	45·9–68·3	Non-comparative
Sundar et al[4]	1994–97	20 mg/kg per d for 30 d	209	73	34·9	28·5–41·8	Non-comparative
Sundar et al[13]	1995–96	20 mg/kg per d for 30 d	50	18	36·0	22·9–50·8	RCT antimony *vs* antimony + interferon γ
		20 mg/kg per d for 15 d + interferon γ 100 mg/m²	50	21	42·0	28·1–56·8	
		20 mg/kg per d for 30 d + interferon γ 100 mg/m²	49	24	49·0	34·4–63·7	
Jha et al[12]	1995	20 mg/kg per d for 30 d	30	19	63·3	43·8–80·1	RCT *vs* aminosidine
Thakur et al[15]	1996	20 mg/kg per d for 30 d	29	20	69·0	49·1–84·7	RCT *vs* aminosidine
Thakur et al[14]	1996	20 mg/kg per d for 30 d	50	26	52·0	37·4–66·3	RCT *vs* aminosidine + antimony
Thakur and Narayan[17]	2000–01	20 mg/kg per d for 28 d	60	28	46·7	33·7–60·0	RCT *vs* amphotericin B

RCT=randomised controlled trial

Table 3: **Clinical studies of antimonials in Bihar, India since 1980**

Reference	Comparison	Standard		Comparator		OR	95% CI	Number of studies	p for χ^2	Risk Difference	NNT
		Cured	Treated	Cured	Treated						
Antimony *vs* paromomycin alone											
Jha et al[12]	Paromomycin 12 mg/kg per d for 21 d	39	59	50	60	0·39	0·16–0·93	2	0·42	−0·17	6
Thakur et al[15]	Paromomycin 16 mg/kg per d for 21 d	39	59	52	57	0·19	0·07–0·55	2	0·46	−0·25	4
	Paromomycin 20 mg/kg per d for 21 d	39	59	54	59	0·18	0·06–0·53	2	0·15	−0·25	4
Antimony *vs* antimony + paromomycin											
Thakur et al[34]	Paromomycin 12 mg/kg per d	26	49	48	52	0·09	0·03–0·30	1	1·00	−0·39	3
	Paromomycin 18 mg/kg per d	26	49	45	48	0·08	0·02–0·28	1	1·00	−0·41	2
Antimony *vs* amphotericin B											
Mishra et al[32]	Amphotericin B 7 mg/kg	25	40	60	60	0·01	0·00–0·24	1	1·00	−0·38	3
Thakur and Narayan[17]	Amphotericin B 20 mg/kg	88	135	115	115	0·02	0·00–0·12	2	0·67	−0·35	3
Thakur et al[33]											
	Total antimony *vs* amphotericin B alone	113	175	175	175	0·02	0·00–0·08	3	0·91	−0·35	
Antimony *vs* interferon γ											
Sundar et al[13]	Antimony 30 d + interferon γ	27	65	37	65	0·53	0·26–1·08	2	0·57	−0·15	7
Sundar et al[13]	Antimony 15 d + interferon γ	18	50	21	50	0·78	0·35–1·74	1	1·00	−0·06	17
Sundar et al[28]											
Amphotericin B deoxycholate *vs* liposomal amphotericin B											
Thakur[34]	Amphotericin B 20 *vs* AmBisome 15 mg/kg total dose	17	17	17	17	NA	NA	1		0·00	
Sundar et al[20]	Amphotericin B 15 *vs* AmBisome 10 mg/kg total dose	49	51	49	51	1·00	0·14–7·39	1	1·00	0·00	
Sundar et al[20]	Amphotericin B 15 *vs* amphotericin B lipid complex 10 mg/kg total dose	49	51	47	51	2·09	0·36–11·93	1	1·00	0·04	−25

Antimony dosage of 20 mg/kg per d for 30 d except for one arm (15 d) in reference 13. NNT=number needed-to-treat; OR=odds ratio

Table 4: **Comparative trials of antimony, paromomycin, and amphotericin B deoxycholate**

All treatments achieved high cure rates and were similarly safe, although it is difficult to compare different studies. A dose-effect relation was apparent in a dose-finding study with total doses of 10 mg/kg, 15 mg/kg, and 20 mg/kg administered over 20 days.[43] Amphotericin B deoxycholate was as active as its liposomal formulations (table 4). Amphotericin B and liposomal amphotericin B (AmBisome, Gilead) had comparable high cure rates at total doses of 20 mg/kg versus 15 mg/kg[34] and 15 mg/kg versus 10 mg/kg,[20] respectively.

AmBisome (476 patients in six studies, two of which were comparative, three dose-finding, and one non-comparative; table 5) was associated with high cure rates even at very low doses. No difference was observed between doses in dose-finding studies in India. The lowest dose of liposomal amphotericin B that has been used for visceral leishmaniasis was a total dose of 3·75 mg/kg, which cured 89% antimony-refractory visceral leishmaniasis.[47] Of particular interest is the finding that a single dose of either 5 mg/kg or 7·5 mg/kg was effective in 91% (95% CI 79–98%) and 90% (95% CI 85–94%) of cases.[24,25] However, information on these regimens is limited to two studies and their sample sizes differ considerably.

Amphotericin B lipid complexes were also effective for re-treating antimony failures.[20,22,23,48,49,50] Efficacy was dose-related in dose-finding studies. Short-course (2 day) regimens were only approximately 80% effective. Results indicate that a total dose of at least 10–15 mg/kg should be administered over 5 days to achieve cure rates of 90% or above.[20] Amphotericin B in fat emulsion seems effective but there is too little data to draw conclusions, with only two studies found in the literature.[21,51]

Amphotericin B deoxycholate is invariably associated with substantial infusion reactions—eg, fever, chills, and tromboflebitis—and occasionally serious toxicity—eg, hypokalaemia, nephrotoxicity, myocarditis, and even death. With lipid formulations of amphotericin B there is substantial improvement in the safety profile of the drug; of the two commercially available preparations tested in Bihar, AmBisome at doses ranging 0·75–15 mg/kg per day produces only minor side-effects (eg, fever, rigor, and backache) in a small proportion of patients. AmBisome was reported to produce an average of 0·6 adverse reactions per treatment, compared with 1·4 with Abelcet and 8·4 with amphotericin B deoxycholate.[20]

In conclusion, prolonged duration of treatment, need for hospitalisation, and infusion-related adverse events are clear handicaps with amphotericin B deoxycholate (figure 2). Lipid-associated formulations require shorter treatments and are much safer, and toxicity is seldom reported with AmBisome.

Pentamidine

Regimens with pentamidine (isethionate or methanosulfonate) were tested in six published and one unpublished trial, all done between the 1980s and 1990s (table 6). No recent studies are available. With one exception, in all studies the drug was administered

Reference	Years of study	Regimen	Number treated	Number cured	Percentage cured	95% CI	Study type
Amphotericin B deoxycholate							
Mishra et al[36]	Not stated	0·5 mg/kg per d for 28 d	15	14	93·3	68·1–99·3	Non-comparative
Mishra et al[37]	1990–91	0·5 mg/kg per d for 14 doses eod	60	59	98·3	91·1–99·9	RCT *vs* pentamidine
Thakur et al[19]	1990–91	1 mg/kg eod for 15 injections	300	298	99·3	97·6–99·3	Non-comparative
Thakur et al[33]	1991	0·05 mg/kg per d to 20 mg/kg total	75	75	100	95·2–100	RCT *vs* antimony
Mishra et al[32]	1992–93	0·5 mg/kg per d for 14 doses eod	40	40	100	91·2–100	RCT *vs* antimony
Jha et al[38]	1993	1 mg/kg eod for 10-15 injections	34	31	91·2	76·3–95·3	Dose-finding (multidrug-resistant)
Thakur et al[39]	Not stated	1 mg/kg per d for 20 d (total 20 mg/kg)	40	40	100	91·2–100	Dose-finding
		0·05–1 mg/kg per d (total 20 mg/kg)	40	40	100	91·2–100	
Thakur et al[40]	1993	1 mg/kg eod (total 20 mg/kg)	60	60	100	94–100	Dose-finding
		0·05 mg increased to 1 mg/kg (total 20 mg/kg)	60	60	100	94–100	
Giri[41]	Not stated	0·75 mg/kg per d for 15 doses eod (total 11·25 mg/kg)	25	25	100	86·3–100	Non-comparative, pentamidine failures
Giri and Singh[42]	Not stated	0·75 mg/kg per d for 15 doses eod (total 11·25 mg/kg)	100	100	100	96·4–100	Non-comparative, antimony failures
Thakur et al[43]	Not stated	1 mg/kg per d for 20 d	96	95	99	94·3–99·9	Dose-finding
		0·75 mg/kg per d for 20 d	96	87	90·6	82·9–95·6	
		0·5 mg/kg per d for 20 d	96	79	82·3	73·1–89·3	
Thakur et al[44]	1995–96	0·05 mg/kg per d increased to 1 mg/kg per d eod (total 20 mg/kg for 43 d)	65	64	98·5	91·7–100	Dose-finding
		1 mg/kg per d for 20 d (total 20 mg/kg for 20 d)	65	64	98·5	91·7–99·9	
Thakur et al[45]	1997	1 mg/kg per d for 20 d	938	931	99·3	98·5–99·7	Non-comparative
Thakur et al[46]	1997–99	1 mg/kg per d for 20 d	7	7	100	59·0–100	Non-comparative, fresh cases, no follow-up data
		1 mg/kg per d for 20 d	266	258	97	94·2–98·7	Antimony + pentamidine resistance
		1 mg/kg per d for 20 d	36	31	86·1	70·5–95·3	Relapsed on amphotericin B treatment
Thakur[34]	Not stated	1 mg/kg per d for 20 d (total 20 mg/kg)	17	17	100	80·5–100	RCT *vs* AmBisome in antimony failure
Sundar et al[18]	1999–2000	1 mg/kg per d for 15 doses eod (total 15 mg/kg)	99	96	97·0	91·4–99·4	RCT *vs* miltefosine
Sundar et al[20]	2001	1 mg/kg eod for 15 injections	51	49	96·1	85–100	RCT *vs* AmBisome/amphotericin B lipid complex
Thakur and Narayan[17]	2000–01	20 mg/kg per d for 20 d	60	60	100	92·5–100	RCT *vs* antimony
AmBisome							
Thakur et al[26]	Not stated	2 mg/kg per d for 7 d (total 14 mg/kg)	10	10	100	69·1–100	Dose-finding
		2 mg/kg per d for 5 d (total 10 mg/kg)	10	10	100	69·1–100	
		2 mg/kg per d for 3 d (total 6 mg/kg)	10	10	100	69·1–100	
Sundar et al[47]	1998	0·75 mg/kg per d for 5 d (total 3·75 mg/kg)	28	25	89·3	71·8–97·7	Dose-finding
		1·5 mg/kg per d for 5 d (total 7·5 mg/kg)	28	26	92·8	76·5–99·1	
		3 mg/kg per d for 5 d (total 15 mg/kg)	28	27	96·4	81·6–99·9	
Sundar et al[24]	1998–99	1 mg/kg per d for 5 d (total 5 mg/kg)	45	42	93·3	82–99	Dose-finding
		5 mg/kg per d for 1 d (total 5 mg/kg)	46	42	91·3	79–98	
Thakur[34]	Not stated	15 mg/kg total dose	17	17	100	80·5–100	RCT *vs* amphotericin B
Sundar et al[20]	2001	2 mg/kg per d for 5 d	51	49	96	85–100	RCT *vs* amphotericin B
Sundar et al[25]	Not stated	7·5 mg/kg per d for 1 d (total 7·5 mg/kg)	203	183	90	85–94	Non-comparative
Amphotericin B lipid complex							
Bodhe et al[48]	Not stated	1 mg/kg per d for 21 d	16	14	87·5	61·7–98·5	Dose-finding
		2 mg/kg per d for 10 d	5	5	100	47·8–100	
		3 mg/kg per d for 7 d	5	4	80	28·4–99·5	
		3 mg/kg per d for 5 d	11	10	90·9	58·7–99·8	
		2 mg/kg per d for 7 d	6	4	66·7	22·3–95·7	
Sundar and Murray[49]	1994	3 mg/kg per d for 10 d (five infusions; total 15 mg/kg)	25	25	100	86·3–100	Non-comparative, antimony failure
Sundar et al[22]	1995–96	1 mg/kg per d for 5 d (total 5 mg/kg)	19	16	84·2	60·4–96·6	Dose-finding, antimony failure
		2 mg/kg per d for 5 d (total 10 mg/kg)	20	18	90·0	60·4–96·6	
		3 mg/kg per d for 5 d (total 15 mg/kg)	21	21	100	83·9–100	
Sundar et al[23]	1996	1·5 mg/kg per d for 5 d (total 7·5 mg/kg)	28	22	78·6	59–91·7	Dose-finding, antimony failure
		2 mg/kg per d for 5 d (total 10 mg/kg)	30	27	90·0	73·5–97·9	
Sundar et al[50]	1997	5 mg/kg per d for 1 d (total 5 mg/kg)	27	19	70·3	50–86	Dose-finding, antimony failure
		5 mg/kg per d at day 1 and 5 (total 10 mg/kg)	24	19	79·1	58–93	
		5 mg/kg per d at day 1 and 2 (total 10 mg/kg)	26	21	80·8	61–93	
Sundar et al[20]	2001	2 mg/kg per d for 5 d	51	47	92	80–98	RCT *vs* amphotericin B
Amphotericin B fat emulsion							
Thakur[51]	1992–93	0·05 to 1 mg/kg per d (total 20 mg/kg)	11	11	100	71·5–100	RCT *vs* standard amphotericin B
Sundar et al[21]	1997–98	2 mg/kg eod (total 10 mg/kg)	70	65	92·9	84·1–97·6	Non-comparative

eod=every other day; RCT=randomised controlled trial

Table 5: **Clinical studies of amphotericin B in Bihar, India since 1990**

Figure 2: **A kala-azar treatment centre in Muzaffarpur, Bihar, with patients receiving amphotericin B infusion as first-line drug**

at 4 mg/kg per day for a variable number of infusions. Efficacy appears to correlate with the number of infusions, although there has been a general decline from the high cure rates of the early 1980s. The concomitant or sequential addition of antimony did not appear to represent a substantial improvement.

Safety is a major concern with pentamidine, with insulin-dependent diabetes mellitus being the most feared and irreversible adverse event.[54,55,57] Such an event, although not uniformly reported, occurs in 4–12% of cases. Shock, myocarditis, and fatal outcomes may be seen, although rarely in visceral leishmaniasis treatment.

Paromomycin

Five trials (431 patients) studied regimens with paromomycin alone (two studies) or combined with antimony (three studies).[12,14,15,58,59] Three of them were comparative against antimony alone (20 mg/kg per day for 30 days), one dose-finding, and one non-comparative (table 7). All comparative and dose-finding studies used computer-generated randomisation; methods to conceal allocation were used in two.[12,15] The doses of 12 mg/kg per day, 16 mg/kg per day, and 20 mg/kg per day for 21 days were tested in two studies with the same protocol in Patna[15] (all doses 86–90% effective, no dose-effect) and Muzaffarpur[12] (16 mg/kg per day and 20 mg/kg per day 93% and 97% effective, respectively). All paromomycin regimens were significantly more effective than antimony at 20 mg/kg per day for 30 days (table 4). Similar results were obtained with the combinations of paromomycin at either 12 mg/kg per day or 18 mg/kg per day and antimony 20 mg/kg per day for 21 days. The drug (alone or combined with antimony) was well-tolerated. Haematology and blood chemistry was checked systematically in these studies; no hepatic or renal toxicity was apparent. No change in hearing was recorded, but audiometry was done on only a fraction of patients. Few cases of hearing disturbance were reported, and all but one were reversible. Gastrointestinal disturbance was reported in few patients receiving the combination.

At the time of writing, a regulatory phase III study with a new formulation has been completed, but the data have yet to be released.

Miltefosine

Six dose-finding studies and one comparative study, with a total of 665 patients, have been published (table 8). Three of these studies identified the dose of 100 mg/kg per day over 4 weeks for further investigation. Toxicity (gastrointestinal, hepatic, and renal) was dose related.

Reference	Years of study	Regimen	Number treated	Number cured	Percentage cured	95% CI	Study type
Jha[52]	Not stated	4 mg/kg per d for >10–12 infusions	82	81	98·8	92·5–99·9	Non-comparative
Thakur[53]	1981	4 mg/kg per d for >15 infusions	92	86	93·4	85·8–97·3	Non-comparative
Jha et al[54]	1983–89	4 mg/kg per d for >20 infusions eod	175	131	75	67·8–81·1	Comparative, non-randomised
		4 mg/kg per d for >20 infusions eod*	65	55	91	81–96·5	
Thakur et al[55]	1988–90	4 mg/kg via infusion 3 times/week until parasite-free (max 40 infusions)	104	80	79·6	67·4–84·3	RCT *vs* pentamidine/antimony
		Pentamidine (as above) + antimony 20 mg/kg per d for 20 d	104	84	80·8	71·6–87·6	
		Pentamidine (as above) then antimony 20 mg/kg per d for 20 d after parasite-free	104	102	98·1	92·6–99·7	
Das et al[56]	1991–97	Pentamidine 2 mg/kg eod + allopurinol 15 mg/kg per d for 30 d	80	73	91·3	82·3–96·1	RCT *vs* pentamidine/allopurinol
		Pentamidine 4 mg/kg eod for 30 d	78	58	74·4	63–83·3	
Mishra et al[37]	1990–91	4 mg/kg per d for 20 infusions eod	60	46	76·7	63·7–86·2	RCT *vs* amphotericin B
Sundar, unpublished	1994–96	4 mg/kg per d for >15 infusions eod	49	33	67	52·5–80·1	Non-comparative

*pentamidine methanesulphonate. eod=every other day; RCT=randomised controlled trial.

Table 6: **Clinical studies of pentamidine in Bihar, India since 1980**

Reference	Years of study	Drug	Regimen	Number treated	Number cured	Percentage cured	95% CI	Study type
Thakur et al[58]	1991	Aminosidine + antimony	12 mg/kg per d + 20 mg/kg per d for 21 d	22	18	81·8	59·7–94·8	Non-comparative
Thakur et al[59]	1992–93	Aminosidine + antimony	12 mg/kg per d + 20 mg/kg per d for 20 d	32	28	87·5	70·1–95·9	Dose-finding
			12 mg/kg per d + 10 mg/kg per d for 20 d	31	22	71·0	52–85·8	Results on day 21, no follow-up
			12 mg/kg per d + 5 mg/kg per d for 20 d	32	23	71·9	53–85·6	
			6 mg/kg per d + 20 mg/kg per d for 20 d	13	9	69·2	38·9–89·6	
			6 mg/kg per d + 10 mg/kg per d for 20 d	12	6	50·0	21·1–78·9	
			6 mg/kg per d + 5 mg/kg per d for 20 d	13	6	46·2	19·2–74·9	
Jha et al[12]	1993–95	Aminosidine	12 mg/kg per d for 21 d	30	23	76·7	57·7–90·1	RCT *vs* antimony
		Aminosidine	16 mg/kg per d for 21 d	30	28	93·3	77·9–99·2	
		Aminosidine	20 mg/kg per d for 21 d	30	29	96·7	81–99·8	
Thakur et al[15]	1996	Aminosidine	12 mg/kg per d for 21d	30	27	90·0	72·3–97·4	RCT *vs* antimony
		Aminosidine	16 mg/kg per d for 21 d	27	24	88·9	69·7–97·1	
		Aminosidine	20 mg/kg per d for 21 d	29	25	86·2	69·7–97·1	
Thakur et al[14]	1996	Aminosidine + antimony	12 mg/kg per d + 20 mg/kg per d for 21 d	52	48	92·3	80·6–97·5	RCT (aminosidine + antimony *vs* antimony alone)
		Aminosidine + antimony	18 mg/kg per d + 20 mg/kg per d for 21 d	48	45	93·8	81·8–98·4	

RCT=randomised controlled trial

Table 7: **Clinical studies of paromomycin in Bihar, India**

Approximately 50% of all patients experienced between one and four episodes of gastrointestinal intolerance over 4 weeks of treatment; vomiting was twice as common as diarrhoea. Although mild in most patients, gastrointestinal toxicity may be severe enough occasionally to require treatment withdrawal. Asymptomatic rises in liver enzyme levels also occurred, but levels recovered spontaneously. Although uncommon, moderate to severe nephrotoxicity was seen in 2% and 1% of patients, respectively, in phase III.[18]

In adults the cure rate was 94% with a daily dose of 100 mg or 50 mg for 4 weeks for individuals weighing more or less than 25 kg, respectively.[18] Cures rates in children were 83–94% with the selected dose of 2·5 mg/kg per day for 28 days.[64,65] Miltefosine has been registered in India since early 2002. The drug cannot be used in women of childbearing age unless contraception is used for the duration of therapy and a further 2 months after because of its teratogenic potential.

Sitamaquine

Sitamaquine (an 8-aminoquinoline) is another candidate for oral treatment, discovered by the Walter Reed Army Institute of Research (Silver Spring, MD, USA) and

Reference	Years of study	Regimen	Number treated	Number cured	Percentage cured	95% CI	Study type
Sundar et al[60]	1998	50 mg eod for 28 d	5	2	40	7·3–83	Dose-finding, definite cure at 8 months follow-up
		100 mg eod for 28 d	5	1	20	1·1–70·1	
		100 mg/d for 28 d	5	5	100	46·3–100	
		150 mg/d for 28 d	5	4	80	29·9–99	
		200 mg/d for 28 d	5	5	100	46·3–100	
		250 mg/d for 28 d	4	4	100	39·6–100	
Sundar et al[61]	1999	100 mg/d for 28 d	17	16	94·1	69·2–99·7	Dose-finding, definite cure at 6 months follow-up
		150 mg/d for 28 d	18	18	100	78·1–100	
		200 mg/d for 28 d	10	10	100	65·6–100	
Jha et al[62]	1999	50 mg/d for 6 weeks	30	28	93·3	76·5–98·8	Dose-finding, definite cure at 6 months follow-up
		50 mg/d for 1 week + 100 mg/d for 3 weeks	30	28	93·3	76·5–98·8	
		100 mg/d for 4 weeks	30	29	96·7	81–99·8	
		100 mg/d for 1 week + 150 mg/d for 3 weeks	30	29	96·7	81–99·8	
Sundar et al[63]	2000	100 mg/d for 2 weeks	18	16	88·9	63·9–98·1	Dose-finding, definite cure at 6 months follow-up
		100 mg/d for 3 weeks	18	18	100	78·1–100	
		100 mg/d for 4 weeks	18	18	100	78·1–100	
Sundar et al[18]	1999–2000	Patient >25 kg: 100 mg/d for 28 d Patient <25 kg: 50 mg/d for 28 d	299	282	94·3	90·9–96·6	RCT phase III; miltefosine *vs* amphotericin B; definite cure at 6 months follow-up
Sundar et al[64]	Not stated	Patient <12 years old: 1·5 mg/kg per d for 28 d	21	19	90·5	69·6–98·8	Dose-finding in children
		2·5 mg/kg per d for 28 d	17	15	88·5	63·6–98·5	
Bhattacharya et al[65]	Not stated	2–11 years old: 2·5 mg/kg per d for 28 d	80	79	94	90·9–96·6	Dose-finding in children

eod=every other day; RCT=randomised controlled trial

Table 8: **Clinical studies of miltefosine in Bihar, India**

under development by GlaxoSmithKline. Progress has been very slow. In Bihar there was a dose-effect in terms of both efficacy and toxicity. The drug was safe up to a dose of 1·75 mg/kg per day for 28 days, except for cyanosis due to methaemoglobinaemia. Although proteinuria was detected in 30% of subjects in whom urinalysis was done, serious toxicity (nephrotoxicity in the form of nephrotic syndrome) occurred in 4% and 7% patients treated with 2 mg/kg and 2·5 mg/kg, respectively, and glomerulonephritis in 7% at 2·5 mg/kg (SS, unpublished data). Efficacy was slightly more predictable than elsewhere:[66] the success rates were 80·6%, 88·9%, 100%, and 80% at daily doses of 1·5 mg/kg, 1·75 mg/kg, 2·0 mg/kg, and 2·5 mg/kg, respectively.

Discussion

We identified, extracted, and evaluated data from a large collection of clinical studies of various treatments for visceral leishmaniasis done in Bihar, India between 1980 and 2004. We believe that this review accurately documents the usefulness of treatments over time in this region, and that it can be used to derive information relevant to other settings. Although we have some reservations on the accuracy of some of the figures derived from individual studies, we are confident of the general trends that emerged.

The database is large (over 7000 patients) and covered a period of 25 years. Several studies occurred when requirements for the conduct and reporting of trials were less demanding. It was in general difficult to extract information on the quality of studies, and in particular on the adequacy of methods used to assign patients to treatment and to conceal allocation. Not all studies provided sufficient information on numbers enrolled and numbers evaluable (intent-to-treat *vs* per-protocol analysis). Safety was unevenly reported. In general, studies reportedly adhered to the then prevalent ethical principles. Most of the studies (72%) compared regimens with different drugs or the same drug (comparative or dose-finding studies).

The characteristics of the regimens considered are summarised in table 2, including standard cost of medication, but without the costs related to hospitalisation and health-care delivery.

For years, Bihar has been facing the problem of untreatable visceral leishmaniasis. Standard first-line antimony has become progressively inadequate. We consider that a central element to this continuous erosion of efficacy has been the use of subtherapeutic doses of antimony because of a combination of policy decisions, substandard drug quality, inadequate prescribing, and poor compliance, compounded by epidemiological features favouring resistance. Although the extent and gravity of the problem is quite unique to antimony in Bihar today, we may witness this same phenomenon where similar conditions occur.

There is evidence that refractoriness to treatment with antimonials is widespread in Bihar, and that clinical failure matches patterns of resistance in vitro and in animal models of patient's isolates.[35,67] Historically, antimony has been used at low doses and unprotected. The trends revealed by this analysis suggest that initially low-dose antimony used alone selected a fraction of the original parasite population with lower sensitivity. Dose escalation over time on a parasite pool with reduced sensitivity was not only unable to catch up with progressive unresponsiveness, but has also further selected parasites with increasing tolerance to higher drug levels. Resistance in India has spread because, by contrast with other endemic areas, the reservoir of infection is human beings, which makes drug resistance recycle quickly because resistant genotypes are not diluted in an animal reservoir. Other areas with anthroponotic transmission would be equally vulnerable.

The quality of Indian antimonials has been questioned in the past. Although generic antimony from India was recently proved to be as good as the branded product,[68–70] this is no guarantee that all products have been consistently good all the time. It is difficult to establish with certainty whether substandard products may have had a role in the generation of resistance, but there is some evidence that it has added to toxicity.[71,72] Independent of quality, a combination of economical constraints, inadequate prescribing, inconvenience due to the prolonged schedule, toxicity, and poor efficacy has likely contributed to the use of subtherapeutic doses. Patients have to use their own resources to buy drugs, but can ill afford it and often consult unqualified practitioners. As little as one in four patients who failed on antimony had adequate treatment, and more than 40% interrupted treatment before completion.[73]

In time, this failure has led to an increase in doses and time of hospitalisation, and the need to retreat failures with rescue drugs; morbidity and the burden of disease have increased, and so have costs to the patients and the health sector. Now we need an antimony replacement. However, neither classic amphotericin B nor pentamidine make a sensible first-line drug. Pentamidine has been virtually abandoned in Bihar because of a combination of serious toxicity, inconvenient schedule of administration, and no efficacy advantage. For quite some time, physicians have relied predominantly on amphotericin B deoxycholate. However, this drug suffers from several limitations: adverse reactions are common, it is much more expensive than sodium stibogluconate, and availability in India is quite erratic.

Efficacious and safe options are needed urgently, and some are available already or are becoming available.[8] This review indicates that treatment policies should consider the use of liposomal amphotericin B, paromomycin, and miltefosine. All three result from

collaborations between the public and private sectors. However, although data are derived from hospital-based studies, in Bihar today only a minority of visceral leishmaniasis patients can access care; we estimate that approximately 12 000–14 000 treatments are delivered each year through the public-health system (eg, primary health centres, district hospitals, and state government medical colleges), while an undetermined number of cases seek treatment outside the public-health sector (approximately 100 000 cases are estimated annually in Bihar). Enlarged coverage and improved access to treatment are major challenges. At present, we are actively collecting data for an in-depth cost-effectiveness analysis of various treatment options in Bihar.

Liposomal amphotericin B (eg, AmBisome) was registered for leishmaniasis with studies done by the public sector coordinated by WHO/TDR: the main advantage is its high effectiveness (prospects for single-dose treatment), the main disadvantage is its high price. In India it is currently available at US$4/mg—ie, almost 3000 times more than sodium stibogluconate and 900 times more than paromomycin. A preferentially low price of $22·3 per ampule (ie, approximately $0·4/mg or one-tenth of the official price) has been obtained by Médecins Sans Frontières, but currently not for use in India. Even then, other costs, notably hospitalisation and injection devices, make the total cost of treatment unaffordable. Therefore, a possible policy would be for all patients to receive a single infusion of 5 mg/kg or 7·5 mg/kg, which would only leave a fraction (up to 20%), of patients needing further treatment. However, we have learnt from antimony that a low dose may help selecting resistant organisms,[27] although, so far, there is no evidence of resistance to amphotericin B. Alternatively, single-dose AmBisome could be combined with a full or shortened course of a companion drug. The high efficacy and fast onset of action of AmBisome would leave a fraction of the original parasite population to be dealt with by the companion drug.

Paromomycin is no longer available in its former parenteral formulation (a powder to be dissolved before injection) that was used in the studies cited here. The registration of a new formulation (a ready-to-use solution for injection) had been on hold for years because of a lack of funding. Funds have now been made available by the Bill and Melinda Gates Foundation to the Institute for One World Health to complete the long overdue remaining trials in collaboration with WHO/TDR. A phase III study has been done in India and the results are expected soon. It is hoped that the drug will be licensed in India by the end of 2005 at a very competitive price (possibly as low as approximately $10 for an adult treatment). The main drawback will be the related to 3 weeks of daily injections, although costs can be reduced by treating patients on an outpatient basis.

Much hope is placed on miltefosine, currently the only oral treatment. The drug, developed jointly by the private (Zentaris, Germany) and the public sector (WHO/TDR and the Indian Council for Medical Research) has received marketing authorisation in India, but not yet for other major foci of the disease. Oral bioavailability is the paradoxical blessing and drawback of miltefosine: it can be used widely on an outpatient basis—thus improving coverage—but this also exposes the drug to misuse. Unregulated use of this drug will have heavy consequences in terms of both its safety and useful therapeutic lifespan.[74]

Because of its teratogenicity potential and long residence time in the body, miltefosine is contra-indicated in women who are pregnant or can not ensure contraception during treatment plus 2 months. We expect approximately one in four visceral leishmaniasis patients to be a woman of childbearing age, and a non-negligible proportion of them not to be eligible to treatment; 2% will be pregnant at the time of diagnosis and an indefinite proportion (potentially up to 30%) could not guarantee not becoming pregnant within 3 months of starting therapy (SS, unpublished data). Furthermore, of those treated, gastrointestinal intolerance will require treatment discontinuation in 3% of cases and substantial nephrotoxicity will occur 1–3% of patients. All of these factors have important implications for a drug that is taken by patients at home.

Thus, in our opinion, miltefosine should not be let loose in the market without adequately educating prescribers and without proper supervision. At present the drug is available only in the private sector at a cost of approximately $145 for the full adult course. A few days' supply of the drug can be purchased through retail chemist shops even without a valid prescription. Patients will not be adequately informed of the contraindications, possible adverse effects, and consequences of not completing treatment. The poor, cash-starved patients of Bihar are buying but a few days' medication and discontinuing it as soon as symptoms abate.[74] Misuse will inevitably expose the drug to resistance[75] and untowards effects. Free supply of the drug through the public sector with directly observed therapy will mitigate the problems and promote better use. At present, distribution and price are being reconsidered. A phase IV study has been done to that effect but only partial results are available.[74]

Although these three treatments feature favourable characteristics, we believe that the evidence is in favour of protecting antileishmanial drugs through combinations, particularly in an area with anthroponotic transmission.[76] Reducing the overall dose and duration of treatment by combining two drugs will cut both direct and indirect costs, which, in India, are incurred mostly by the patient. If one or both components of the combination were oral, then hospital stay would be limited to the initial few days of assessment and start of therapy, which could then be continued at home with patients returning for weekly supply and supervision.

The short course will favour adherence to the prescribed regimen, particularly if treatment acts rapidly and patients feel better within a few days. In addition, this will broaden the treatment base, which is currently limited by bed capacity, among other things (figure 3). There is evidence from other infectious diseases (eg, tuberculosis, HIV/AIDS, malaria) that resistance is less likely to occur when two drugs acting on distinct targets are used simultaneously.

Several factors should be considered in identifying drugs suited for coadministration, including pharmacological (pharmacokinetic and pharmacodynamic characteristics, possible interactions) and practical considerations. We have imperfect experimental models to identify companion drugs and a short list of drugs to select from. Paromomycin plus antimony has proved effective and safe already, although this combination may not be a long-term solution where the level of antimony resistance is high. Other combinations should be tried, including miltefosine, paromomycin and, cost permitting, single-dose liposomal amphotericin B. The availability of another oral treatment like sitamaquine would make it possible to test a fully oral combination therapy with miltefosine. It is clear that sitamaquine has substantial antileishmanial activity, and should be developed further and much quicker than in the past. Larger clinical trials are needed with selected doses to better define its role. It is also important that, to identify candidate combination therapies, the different treatments are carefully assessed for their cost-effectiveness. There is an obvious need to intensify research to discover more antileishmanial compounds so that we have enough in reserve in case the existing drugs fail. Candidates should be assessed more quickly and thoroughly: the development of paromomycin and sitamaquine has been very slow; limited information on the use of AmBisome in visceral leishmaniasis was available at the time it was registered. Reasons for this are mostly due to the low priority, little funding, and the extent of neglect of this disease. Implications in terms of public health and individual suffering are of great consequence. Paromomycin could have replaced or complemented the then failing antimony some 8–10 years ago; AmBisome was available but the price barrier impeded its use. It is only too enticing to attempt to quantify the amount of suffering and costs that would have been averted.

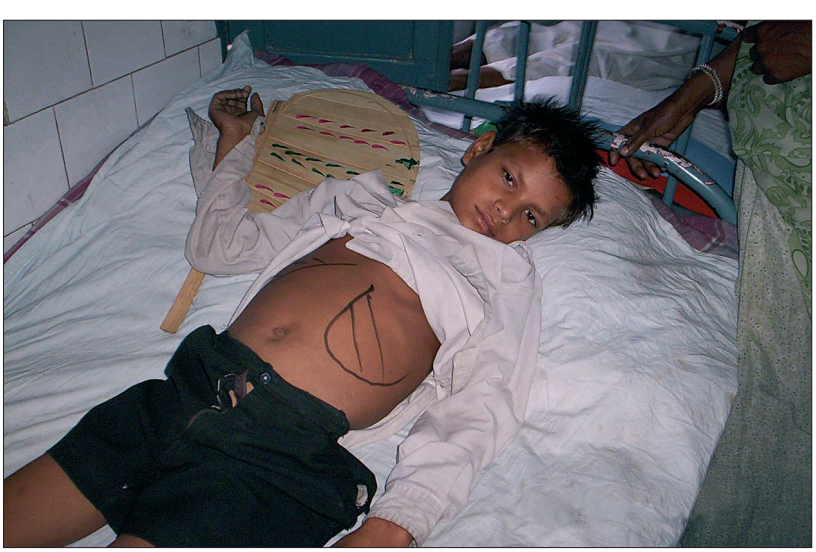

Figure 3: **A sick child with hepatosplenomegaly in a crowded ward**

Search strategy and selection criteria

These are described in the Methods section.

From the number of studies identified, it is reassuring to see that treatment effects have been intensely monitored in Bihar, and alternative treatment options actively sought. However, quality of studies vary, safety is under reported, and limited data exist for some regimens. Some such regimens deserve more studies. Safety information is essential for policy making; we would encourage both investigators and publishers to improve and standardise reporting on tolerabilty and toxicity in clinical trials. Also, we strongly advocate continuous, active pharmacovigilance when new drugs are deployed (as will be the case for miltefosine and paromomycin) to document safety, efficacy, and appropriate use.

Finally, we believe that the lessons learnt here could inform and guide future interventions, both regionally (the planned elimination of visceral leishmaniasis as a public-health problem in the Indian subcontinent) and elsewhere.

Conflicts of interest

We declare that we have no conflicts of interest.

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