Aminosidine plus sodium stibogluconate for the treatment of Indian kala-azar: a randomized dose-finding clinical trial

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Abstract

This randomized, open sequential design trial was set up to assess the efficacy, tolerability and toxicity of 20 d courses of combined intramuscular aminosidine and sodium stibogluconate at various dosages in patients with newly-diagnosed kala-azar in Bihar, India. Three successive studies of 96 patients each were originally planned with aminosidine administered at 12, 6 and 3 mg/kg/d, respectively. For each aminosidine dosage, patients were randomly assigned to receive sodium stibogluconate at 20, 10 or 5 mg/kg/d of antimony. Ninety-six patients were enrolled and assigned aminosidine 12 mg/kg/d as scheduled. In the subsequent study with aminosidine at 6 mg/kg/d, the trial was interrupted after 40 patients had entered owing to inadequacy of the treatment. With aminosidine 12 mg/kg/d the success rates with sodium stibogluconate at 20, 10 and 5 mg/kg/d were 88%, 71% and 72%, respectively and did not differ significantly. With aminosidine 6 mg/kg/d, 69%, 50% and 46% of patients were cured with the same sodium stibogluconate doses, respectively; again, there was no significant difference between the subgroups. The overall success rate with aminosidine at 12 mg/kg/d (76%) was significantly higher than that with 6 mg/kg/d (55%) (odds ratio=2·69; 95% confidence interval, 1·11–6·4). Patients improved clinically and the treatments were equally well tolerated. The combination of aminosidine 12 mg/kg/d and sodium stibogluconate 20 mg/kg/d for 20 d appears to be an effective and safe replacement in Bihar for sodium stibogluconate alone for ≥40 d.

Keywords: visceral leishmaniasis, *Leishmania donovani*, aminosidine plus sodium stibogluconate, treatment, India

Introduction

Response to treatment with organic pentavalent antimonials, the standard first-line treatment for kala-azar (visceral leishmaniasis), has been decreasing over the years in India (INDIA, 1991; THAKUR, 1993). Several papers have reported prolonging courses and increasing dosages in an attempt to compensate for diminishing activity (PETER, 1981; THAKUR, 1984; THAKUR *et al.*, 1988, 1991). Now there is hardly any patient with kala-azar in Bihar who needs fewer than 40 d of sodium stibogluconate at 20 mg/kg/d. Despite such prolonged treatment, the success rate is at present only slightly above 80% and it is well below 70% after 3 weeks of treatment. However, adverse reactions are seen more frequently and costs have increased dramatically (THAKUR *et al.*, 1991; OLLIARO & BRYCESON, 1993).

It is therefore necessary to increase efficacy and also reduce the total drug intake. A probable solution seemed to be combining sodium stibogluconate with aminosidine, which is available as an injectable formulation (Gabbromycin®, Farmitalia Carlo Erba). Aminosidine is an aminoglycosidic aminocyclitol antibiotic belonging to the 2-deoxystreptamine subgroup (ARCAMONE *et al.*, 1975). Identity of the catenulin–neomycin antibiotics, including aminosidine and paromomycin, produced by different *Streptomyces* species, was first recognized by SCHILLING & SCHAFFNER (1961). Although the antileishmanial properties of paromomycin (=aminosidine) were first shown by NEAL in 1968, only recently was the drug used in patients with kala-azar (CHUNGE *et al.*, 1990). As this study indicated that the combination of aminosidine and sodium stibogluconate was superior to either drug alone, we did a pilot study to assess the applicability of those results to Indian kala-azar (THAKUR *et al.*, 1992); a 20 d course of aminosidine at 12 mg/kg/d and sodium stibogluconate at 20 mg/kg/d was 82% effective and was well tolerated. The efficacy of combined regimens of aminosidine and antimony has been further documented in visceral leishmaniasis due to *Leishmania donovani* and disseminated cutaneous leishmaniasis due to *L. aethiopica* by SEAMAN *et al.* (1993) and TEKLEMARIAM *et al.* (1994).

A dose-finding study was therefore set up to confirm these encouraging results and to assess the efficacy and safety of different ratios of aminosidine and sodium stibogluconate.

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Methods

Study design and treatment schedules

This was a randomized, open, sequential-design trial. The protocol received ethical clearance by the Indian Drug Controller and the local Ethical Committee. After giving informed consent, patients were enrolled, admitted to hospital and treated at Tripolia Social Service Hospital, Patna, Bihar, India. The study drugs were aminosidine (Gabbromycin®, Farmitalia Carlo Erba, Milano, Italy) and sodium stibogluconate (Stibanate®, The Gluconate Ltd, Calcutta, India).

The trial was designed to include 3 successive groups of 96 patients, each receiving aminosidine at 12 mg/kg/d (group 1), 6 mg/kg/d (group 2), or 3 mg/kg/d (group 3), but the last dosage was not used (see below). Within each study, patients were randomly assigned to 3 subgroups receiving 20 mg/kg/d (subgroup *a*), 10 mg/kg/d (subgroup *b*), or 5 mg/kg/d (subgroup *c*) of sodium stibogluconate. The efficacy of each treatment schedule was to be assessed on the basis of the response rate (p), defined as the proportion of patients with negative spleen aspirate at the end of therapy (day 21). The treatment was to be considered ineffective if $p \leq 0{\cdot}65$. Assuming an α level of 0·05 and a power $(1-\beta)$ of 0·8 to detect $p \geq 0{\cdot}85$, it was calculated that 32 patients were required for each dose combination. The trial was to start with patients assigned to aminosidine at 12 mg/kg/d (group 1). If a group was successfully treated, a further group of 32 patients was to be enrolled and treated with the same dose of sodium stibogluconate and half the dose of aminosidine (i.e., 6 mg/kg/d; group 2). Assignment to the sodium stibogluconate dose within each trial (subgroups *a*, *b* or *c*) was randomized.

Both drugs were administered intramuscularly at different sites for 20 d. Patients were admitted to hospital the day before treatment and remained in hospital for 20 d treatment and post-treatment assessment on day 21. Follow-up visits were scheduled for 90 d and 180 d after the end of treatment.

Patients

Only patients with previously untreated kala-azar, diagnosed by finding amastigotes in splenic aspirate and the presence of typical clinical signs and symptoms of kala-azar were included. All patients came from the same geographical area of India (Vaishali, Samastipur, Musaffarpur and Patna districts).

Clinical and laboratory examinations

Clinical and laboratory investigations were carried out before starting treatment (baseline values), on days 7 and 14 during treatment, one day after end of treatment (day 21), and during the drug-free follow-up period on days 90 and 180. The examinations included: (i) splenic aspirate to assess parasite load according to CHULAY & BRYCESON (1983) and measurement of spleen and liver size (from costal margin along the anterior axillary line); (ii) body weight and body temperature; (iii) white blood cell (WBC) counts and haemoglobin determination; and (iv) other haematological and blood chemistry tests, including liver and renal function tests. Electrocardiography (ECG) and audiometry were scheduled before and after treatment.

Clinical laboratory values were classified as normal or abnormal according to whether they were within or outside the normal ranges. To assess the direction of changes, the paired observations of values classified as described above were analysed at the baseline and on day 21 by using χ^2 or Fisher's exact two-tailed test (as appropriate) for each treatment group. A stratified analysis was also conducted within each phase and the Mantel-Haenszel weighted OR and 95% CI were calculated.

All results were assessed at a significance level of $P=0{\cdot}05$.

Results

Aminosidine at 12 mg/kg/d (group 1)

The 3 subgroups did not differ with respect to demographic or baseline characteristics (Table 1).

Table 1. Baseline characteristics of kala-azar patients treated with aminosidine and sodium stibogluconate

	Aminosidine dosage 12 mg/kg/d			6 mg/kg/d		
	Mean	SD	Range	Mean	SD	Range
Age (years)	24·8	14·1	5–67	22·5	14·9	6–66
Weight (kg)	37·8	13·4	11–72	33·2	12·2	12–56
Temperature (°C)	37·2	1·4	36·5–40	37·8	1·4	36·7–39·4
Parasite grading	2·9	1·2	1–5	3·2	1·3	1–6
Spleen size (cm)	8·3	3·4	2–16	7·7	3	2–15
Liver size (cm)	3·9	2	0–10	4·1	1·9	1–8
Haemoglobin (g/dL)	8·7	2·2	3–12·4	8·7	1·9	3·8–11·6
White blood cells ($\times 1000/mm^3$)	3436·6	1280·7	1350–8000	3758·8	1355·6	1050–6800

Efficacy

Effective treatment was defined as a negative splenic aspirate at the end of therapy (day 21). Upon discharge, patients were advised to report for follow-up on days 90 and 180 to monitor the occurrence of relapses and late cures. However, only a descriptive analysis was possible at follow-up due to the low numbers of patients reporting.

Test of cure. Treatment 1*a* gave the highest proportion of patients with negative splenic aspirate at the end of therapy, though the result was not statistically significantly superior to those obtained in the other groups (Table 2). The proportion of patients with a negative splenic aspirate increased with the length of treatment with all 3 regimens (Table 2).

Table 2. Success rates (conversion from positive to negative splenic aspirate) for kala-azar patients treated with aminosidine and sodium stibogluconate

	No. of negative patients/no. examined			
	Subgroup *a*[a]	Subgroup *b*[a]	Subgroup *c*[a]	Total
Aminosidine dosage				
12 mg/kg/d (group 1)				
Baseline	0/32	0/32	0/32	0/96
Day 7	6/32 (19%)	10/32 (31%)	6/32 (19%)	22/96 (23%)
Day 14	18/32 (56%)	17/32 (53%)	17/32 (53%)	52/96 (54%)
Day 21	28/32 (88%)	22/31 (71%)	23/32 (72%)	73/95 (76%)
6 mg/kg/d (group 2)				
Baseline	0/13	0/13	0/14	0/40
Day 7	1/13 (8%)	2/13 (15%)	1/13 (8%)	4/39 (10%)
Day 14	3/10 (30%)	4/11 (36%)	3/13 (23%)	10/34 (29%)
Day 21	9/13 (69%)	6/12 (50%)	6/13 (46%)	21/38 (55%)

[a]Sodium stibogluconate dosages: subgroup *a*, 20 mg/kg/d; subgroup *b*, 10 mg/kg/d; subgroup *c*, 5 mg/kg/d.

Data handling and analyses

Data were recorded on individual case record forms, entered into a Lotus® 123 computer package, verified and checked, and then transferred to EpiInfo for analysis.

The proportions of patients with negative spleen aspirates were compared within and between each group by calculating χ^2; odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. The distribution of 'cures' by visit was assessed and the results compared with the aid of the Mantel–Haenszel test. A stratified analysis was conducted to compare the success rates in the 2 aminosidine groups and OR and 95% CI were calculated.

Paired observations of spleen and liver size and haematological and blood chemistry data were compared at the baseline and on day 21 using the Kruskall-Wallis *H* test.

Parasite load decreased in all patients but one in group 1*b*; no significant difference was apparent between the subgroups.

Overall, 47 (49%) and 26 (27%) patients reported for follow-up visits on days 90 and 180, respectively. Of these, 38 and 21 had been classified as treatment successes on day 21. A relapse occurred in 2 patients in group 1*a* (one on day 90 and one on day 180), one in group 1*b* on day 90 (who became negative again on day 180); and 2 in group 1*c* (one on day 90 and one on day 180).

Conversely, of the 48 patients who reported for at least one follow-up visit, 9 who had been 'failures' at the end of therapy subsequently provided a negative spleen aspirate: 2 each in groups 1*a* and 1*c*, and 4 in group 1*b*.

Clinical assessment. Most patients improved clinically on treatment. Considering all patients, paired observations showed a significant decrease in spleen size from

8·3 cm (standard deviation [SD] 3·4) to 4 cm (SD 3) ($P<0·001$) and a substantial, though non-statistically significant, decrease in liver size (from 3·9 cm (SD 2) to 0·5 cm (SD 1)).

All patients had a palpable spleen before starting treatment; in 11 of them, the spleen was no longer palpable by the end of treatment, with no difference between groups. Comparing paired measurements of spleen size at baseline and on day 21, a statistically significant reduction was observed in treatment groups 1*a* ($P=0·03$) and 1*b* ($P=0·02$), but not in group 1*c* ($P=0·1$). However, no statistically significant difference in spleen size reduction was observed between the groups.

Similar findings were obtained with liver size. No statistically significant difference was found in or between the groups when comparing baseline and day 21. At baseline, 6 patients, 3 in group 1*a*, 2 in group 1*b* and one in group 1*c*, had a non-palpable liver. On day 21, the liver was not palpable in 24, 27 and 21 patients, respectively.

Most patients were afebrile by the end of therapy. All had intermittent fever before starting treatment, although a proportion of them had no fever at baseline.

Clinical laboratory results. Overall, WBC counts increased from a mean of $3436·6\times10^3$/mm^3 (SD $1280·7\times10^3$, range 1340×10^3–8000×10^3) at the baseline to $4810·6\times10^3$/mm^3 (SD $1678·37\times10^3$, range 2000×10^3–10400×10^3) at the end of therapy. No statistically significant difference was apparent among the groups when paired observations before and after treatment were compared for each patient. However, there were significantly more patients with WBC counts within the normal limits after treatment than at the baseline (OR=15·52; 95% CI 32·14–60·09). Almost all patients had low haemoglobin values at baseline and they remained low after therapy. Total protein increased significantly on treatment from 8·9 g/dL (SD 1·7) at baseline to 9·2 g/dL (SD 1·5) at the end of therapy. However, most patients had normal pre-treatment values.

Tolerability. All 3 regimens were equally well tolerated, and with no patient was treatment discontinued due to an adverse event.

Of the 96 patients treated, 11 (11%) experienced 15 adverse events. Four patients (13%) in group 1*a* reported 5 adverse events: 3 affected the gastrointestinal system (abdominal pain, nausea and gastrointestinal haemorrhage), one the nervous system (giddiness), and one was a breast abscess. Two patients (6%) in group 1*b* reported 3 adverse events: one abdominal pain, one gastroenteritis and one case of fever. Five patients (16%) in group 1*c* reported 7 adverse events: 3 cases of abdominal pain, one of vomiting, 2 cases of hearing disturbance and one case of herpes infection. One patient in groups 1*a* and 1*b* and 2 patients in group 1*c* reported 2 adverse events each. Only one adverse event was reportedly severe but not drug-related; this occurred in group 1*a*.

No renal toxicity was apparent. At the baseline, most patients had creatinine and blood urea nitrogen (BUN) values falling within the normal limits; post-treatment values of creatinine and BUN remained unchanged from the pre-treatment values. The mean creatinine level was 1·02 mg/dL (SD 0·57) at the baseline and 0·79 (SD 0·36) after treatment; the corresponding values for BUN were 10·07 mg/dL (SD 4·56) and 10·1 mg/dL (SD 3·83).

A significant increase in serum levels was observed for liver enzymes (alanine and aspartate aminotransferases, ALT and AST) in the paired observations. Also, significantly more abnormal values were observed on day 21 than at the baseline. The ORs for increased values after treatment were 3·69 (95% CI 1·35–10·05) for ALT and 4·65 (95% CI 1·87–11·55) for AST.

No abnormality in the ECG and no change in hearing was found after treatment, although audiometry was performed in only about 10% of the subjects.

Aminosidine at 6 mg/kg/d (group 2)

Patient enrolment was stopped after 40 patients had entered the study, owing to inadequacy of the treatment. The patient's baseline characteristics (Table 1) did not differ significantly between the subgroups.

Test of cure. The success rate at the end of therapy was marginally better in group 2*a* than in groups 2*b* and 2*c* (Table 2).

Twenty-four and 12 patients were followed up for 90 and 180 d, respectively; 13 and 7 were classified as treatment success at the end of therapy. A relapse occurred on day 90 in one patient in group 2*c* and one in group 2*a*, and on day 180 for one patient in group 2*a* and one in group 2*b*.

Of the 24 patients with at least one follow-up visit, 5 treatment failures on day 21 (2, 4 and one in groups 2*a*, 2*b* and 2*c*, respectively) had negative spleen aspirates after completion of their treatment.

Clinical laboratory results. WBC counts and haemoglobin levels increased, though not significantly, during treatment: mean WBC counts were 3758·75 cells/mm^3 (SD 1256·6) at the baseline and 4384·2 (SD 1684·15) at the end of therapy; haemoglobin values were 8·68 g/dL (SD 1·86) at the baseline and 10·2 g/dL (SD 1·64) on day 21. No difference was apparent between the treatment subgroups.

Tolerability. Four adverse events occurred, 2 in group 1*a* and 2 in group 1*c*. No statistically significant difference was detected between the paired observations at the baseline and after treatment for all variables tested.

Between-study comparison (aminosidine 12 mg/kg/d versus 6 mg/kg/d)

Patients assigned to receive aminosidine at 6 mg/kg/d were similar to those enrolled in the 12 mg/kg/d study (Table 1).

The overall cure rate at end of therapy was 76% (73/95) with aminosidine at 12 mg/kg/d, while it was 55% (21/38) with aminosidine at 6 mg/kg/d (Table 2). The difference in cure rates was statistically significant (χ^2 test, $P=0·014$). Overall, patients receiving aminosidine at 12 mg/kg/d had a 2·69 (95% CI 1·1–6·4) better chance of having a negative splenic aspirate at the end of therapy than did those treated with 6 mg/kg/d. Similarly, at the end of therapy, stratified analysis of the numbers with negative aspirates among those receiving aminosidine at 12 mg/kg/d compared with the numbers among those receiving 6 mg/kg/d gave an OR=2·81 (95% CI 1·15–6·91) ($P=0·022$).

By contrast, comparison of distribution of negative aspirates at successive examinations in the group receiving aminosidine at 12 mg/kg/d with that among those receiving 6 mg/kg/d showed substantial, though non-significant, differences. The relevant ORs were 3·07 (95% CI 0·95–10·47) with sodium stibogluconate at 20 mg/kg/d, 2·17 (95% CI 0·71–6·8 9) with sodium stibogluconate at 10 mg/kg/d, and 2·8 (95% CI 0·9–9·02) with sodium stibogluconate at 5 mg/kg/d.

No difference was observed in the incidence of adverse events in patients receiving the 2 aminosidine dosages, nor was any dose-related toxicity apparent with either drug. Treatment was generally acceptable in spite of the quite large volumes injected intramuscularly.

Discussion

The aim of this trial was to assess whether a combination of aminosidine and antimony for a shorter duration could substitute for conventional, prolonged antimony and if so what were the limits of the combination in terms of efficacy and safety. The trial was therefore designed to identify effective and safe drug regimens and the sample size was calculated so as to enable identification of major differences among treatments.

Aminosidine alone is active *in vitro* on *L. donovani*, with no cross-resistance with antimonial drugs: the two drugs act synergistically (S. Croft, personal communication). However, aminosidine and sodium stibogluconate were only additive when tested *in vivo* in a mouse model

(McCoy & Neal, 1989). The antileishmanial action of aminosidine is thought to be related to misreading of messenger ribonucleic acid (Edlind, 1991); the target of pentavalent antimonials is also not known precisely, although various mechanisms have been suggested (Berman, 1988). At present, there is therefore no clear explanation for the synergistic action of these drugs *in vitro*. The slightly dissimilar results obtained in the experiments *in vivo* (McCoy & Neal, 1989), and partly confirmed by our study, were probably due to the different pharmacokinetic profiles of the 2 drugs.

Combined with antimony, aminosidine at 12 mg/kg/d was significantly more effective than at 6 mg/kg/d. By contrast, no difference was apparent between the various doses of sodium stibogluconate. When combined with aminosidine at either dose, some efficacy was seen even with 5 mg/kg/d of sodium stibogluconate for 20 d, a dose regimen that would be devoid of efficacy if given alone. When planning the experiment, we had decided to reject any dosage which was not 65% or more effective. Although aminosidine at 6 mg/kg/d + sodium stibogluconate at all doses used proved effective in 69% of cases, it was thought to be ethically necessary to close the study of this halved dose of aminisodine as the patients were clearly being offered less efficacious therapy.

The aminosidine 12 mg/kg/d + sodium stibogluconate 20 mg/kg/d regimen is preferred because, despite not achieving statistical significance, the results indicated that it was more active and no less safe than the other regimens. There was no obvious difference between the groups with respect to the other indicators of efficacy which we evaluated.

Combination therapy with aminosidine has been tested clinically in 2 studies in Africa (Chunge *et al.*, 1990; Seaman *et al.*, 1993) and in our pilot study in India (Thakur *et al.*, 1992). In both the Kenya and Sudan trials aminosidine was administered at 15 mg/kg/d in conjunction with sodium stibogluconate at 20 mg/kg/d for 14 and 15–17 d, respectively. The cure rates at the end of therapy were 100% and 95%, respectively. A drug-free follow-up was performed in Kenya, assessing the definite cure rate at 87%. In both studies, the combination therapy was significantly more effective than sodium stibogluconate alone.

In Bihar, the cure rate at the end of therapy was 82% in the pilot study, and 88% both at the end of therapy and at follow-up with the maximum dose tested, aminosidine 12 mg/kg/d and sodium stibogluconate 20 mg/kg/d for 20 d, in the current study. These results were remarkably consistent and similar to those obtained in Kenya with 2 weeks' treatment including aminosidine at 15 mg/kg/d. However, the cure rate in Bihar after 2 weeks of therapy was only 56% at best. It seems, therefore, that treatment for 14–17 d with 15 mg/kg/d of aminosidine combined with 20 mg/kg/d of sodium stibogluconate is highly effective in African kala-azar but would be less likely to succeed in India.

The combination of 14 mg/kg/d aminosidine with sodium stibogluconate 10 mg/kg/d has also proved effective and safe in a limited number of patients with diffuse cutaneous leishmaniasis caused by *L. aethiopica* (see Teklemariam *et al.*, 1994).

Treatments were well tolerated and no dose-related toxicity was apparent. Only liver enzymes rose slightly on treatment, which is a probable sign of antimony toxicity. However, these 20 d courses with sodium stibogluconate did not result in any cardiac toxicity, which highlights an additional benefit of combining antimony and aminosidine and curtailing therapy. It is unfortunate that, for technical reasons, post-treatment audiograms could be performed on only a limited number of patients; the available data do not suggest ototoxicity.

The principal flaw of this study might be seen in the drug-free follow-up. Despite contacting patients, travelling and general inconvenience prevented many from reporting for the scheduled assessments. The late 'cures' and relapses observed indicate the need for more stringent planning of follow-up visits in future studies, including provision of financial support for travelling and lost income. However, even with all the weaknesses of follow-up in the present study, the overall conclusions did not differ after including the available information on relapses and late successes.

Based on our results and those reported elsewhere, we therefore conclude that at least 12 mg/kg of aminosidine should be given daily for 20 d when combined with the standard dose of sodium stibogluconate for the treatment of Indian kala-azar. Under the conditions of our study this regimen appears to be highly effective and well tolerated.

Further studies supported by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases are being conducted in order to assess the efficacy and safety of aminosidine alone. Another study is being planned to compare the combined treatment with standard antimonial therapy in the same patient population.

Acknowledgements

We are grateful to Professor L. Donno for his invaluable help in designing and setting up this study and to Ms A. Maniero, Professor C. Praga, Dr S. Gothokar and Mr U. Khaul (Farmitalia Carlo Erba) for assistance in the preparation and follow up of the study. We also thank Sister Clementina, Tripolia Social Service Hospital, for her good care of the patients. The contribution of Dr A. Bryceson (Hospital for Tropical Diseases, London, UK) to the aminosidine programme is also warmly acknowledged. We are indebted to Mrs L. Baldry and Dr F. Neva (NIH) for reviewing the manuscript.

This study was supported by a grant from Farmitalia Carlo Erba, Milano, Italy. At the time the study was set up, P. Olliaro was employed as a scientist at R & D Anti-infectives, Farmitalia Carlo Erba, Milano, Italy (now Pharmacia–Farmitalia Carlo Erba).

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Received 20 July 1994; revised 13 September 1994; accepted for publication 21 September 1994

Announcements

The Basics of Travellers' Health

Swiss Tropical Institute Basel and the International Society for Tropical Medicine

This short certificate course will be held in Basel from *26–29 June 1995*. It is intended to prepare professionals (doctors, senior nursing staff, educators, teachers, trainers and travel industry staff) to give advice to travellers and on the treatment of travel-related diseases, with the main emphasis on the tropics.

The course will be taught in English and the fee is Swiss francs 550.–

Applications must be received by 31 May 1995. Further information can be obtained from Mrs M. Slaoui, Swiss Tropical Institute, Socinstrasse 57, P.O. Box, CH-4002 Basel, Switzerland; phone +41 61 284 82 80, fax +44 61 271 79 51, telex 96 25 08.

Gender, Health and Technology

Entries are required for the fifth **IDRC/TDR** award on gender and tropical diseases. The award (Canadian $5000) will be given to the author or authors of the best paper on this subject. Ideally, the papers should focus on one or more of the TDR's target diseases; *they must be original and not previously published*, but may be based either on secondary sources or original research. Previous winners of an IDRC/TDR award are *not* eligible.

Papers must be written in English, French or Spanish and must have a brief summary in English; they should not exceed 30 typed, double-spaced A4 pages and must be submitted by *30 April 1996*. It would be helpful if intending authors informed Dr Carol Vlassoff (address below) well in advance of the deadline.

Papers should be submitted to Dr Carol Vlassoff, Manager, Gender and Tropical Diseases Task Force, TDR/WHO, 1211 Geneva 27, Switzerland.