Entre septembre 2003 et avril 2004, l'approvisionnement de l'hôpital d'Amudat, Ouganda, en antimonials fut interrompu et tous les cas de leishmaniose viscérale durent être traité par amphotéricine B déoxycholate (AmB). Ceci permit d'évaluer la sécurité et l'efficacité de l'AmB, par comparaison avec une cohorte historique de patients traités dans le même hôpital par méglumine antimoniate (SbV). Le taux de létalité hospitalier fut de 4.8% (IC95% =2.4%-8.8%) parmi les 210 de patients traités par AmB et de 3.7% (IC=1.4%-7.9%) parmi les 161 patients traités par SbV (p>0.20). Les événements indésirables nécessitant l'interruption du traitement furent rares dans les deux groupes. Les échecs de traitement (à savoir non-réponses et rechutes) représenteront 2.9% (IC=1.2%-6.4%) des patients traités par AmB et 1.2% (IC=0.1%-4.4%) des patients traités par SbV (p>0.20). L'AmB a une efficacité et un profil de sécurité similaires à SbV pour le traitement de la leishmaniose viscérale en Ouganda.


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Safety and effectiveness of amphotericin B deoxycholate for the treatment of visceral leishmaniasis in Uganda

Y. MUELLER*, A. NGUIMFACK*, P. CAVAILLER†, S. COUFFIGNAL*, J. B. RWAKIMARI‡, L. LOUTAN§ and F. CHAPPUIS*§

*Médecins Sans Frontières, Swiss Section, Rue de Lausanne 78, 1202 Geneva, Switzerland
†Epicentre, 8 Rue Saint-Sabin, 75011 Paris, France
‡Ministry of Health of Uganda, Plot 6 Lourdel Road, Wandegeya, P.O. Box 7272, Kampala, Uganda
§Travel and Migration Medicine Unit, Geneva University Hospital, Rue Micheli-du-Crest 24, 1211 Geneva 14, Switzerland

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Between September 2003 and April 2004, the supply of antimonial drugs to Amudat Hospital, in north–eastern Uganda, was interrupted and all cases of visceral leishmaniasis presenting at the hospital could only be treated with amphotericin B deoxycholate (AmB). This allowed the safety and effectiveness of the AmB to be evaluated, in comparison with an historical cohort of patients treated, at the same hospital, with meglumine antimoniate (SbV). Demographic and clinical data were collected before and after treatment. Adverse effects were recorded passively in all the subjects, and actively, using a standardized questionnaire, in a sub-group of the patients given AmB. The in-hospital case-fatality ‘rates’ were 4.8% [95% confidence interval (CI) 2.4%–8.8%] among the 210 patients treated with AmB and 3.7% (CI 1.4%–7.9%) among the 161 patients treated with SbV (P > 0.20). Adverse effects requiring treatment interruption were rare in both cohorts. Treatment failures (i.e. non-responses or relapses) were observed in 2.9% (CI 1.2%–6.4%) of the patients treated with AmB and 1.2% (CI 0.1%–4.4%) of the patients treated with SbV (P > 0.20). For the treatment of visceral leishmaniasis in Uganda, AmB therefore had a similar effectiveness and safety profile to that of meglumine antimoniate.

Human visceral leishmaniasis (VL), or kala-azar, is a life-threatening haemo-protozoal disease, with an estimated 500,000 new cases occurring every year world-wide (WHO, 1997). Treatment of VL remains a major problem, especially in resource-poor areas (Guerin et al., 2002). Patients’ access to branded pentavalent antimonial drugs, such as meglumine antimoniate (Glucantime™; Aventis, Strasbourg, France) or sodium stibogluconate (Pentostam™, GlaxoSmithKline, Brentford, U.K.) is limited, mainly because of high costs. The generic form of sodium stibogluconate (SSG), as manufactured by Albert David Ltd in Calcutta, India, has substantially reduced the cost of treatment and appears similar, in terms of safety and efficacy, to the branded drugs (Veeken et al., 2000; Moore et al., 2001; Ritmeijer et al., 2001; Bermúdez et al., 2006). Unfortunately, this generic SSG is still not authorized for importation into many countries. In the treatment of VL, amphotericin B, either in its conventional form (amphotericin B deoxycholate) or liposomal form, is a useful alternative to antimonial drugs (Sundar et al., 2004). Amphotericin B deoxycholate (AmB) is now used as a first-line drug in the Indian
state of Bihar, where resistance to antimonials has become a major problem (Mishra et al., 1994; Thakur and Narayan, 2004), and as a second-line drug in some Latin American (Santos et al., 2002) and Asian countries, such as Nepal (Rijal et al., 2003). In India, AmB has been found to be effective against VL and safe, even in rural settings (Thakur and Ahmed, 2001). Although AmB is not yet licensed for the treatment of VL in any country in the world, it is (unlike generic SSG) widely available as it is licensed for the treatment of fungal infections. There are no published data on the use of AmB against VL in Africa and it would be dangerous to assume that the drug will be as safe and effective against VL in Africa as it is against the disease in India. In Africa, the drug sensitivities of the parasites involved and the relevant characteristics of the human hosts probably differ from those in India (Berman et al., 1998). Although liposomal amphotericin B (AmBisome™; Gilead Sciences, Foster City, CA), which is even better tolerated and more practical to use than conventional AmB (Sundar et al., 2004), is used for the first-line treatment of VL in developed countries, it remains too expensive for most patients in the developing world (Bern et al., 2006).

In Uganda, VL is only known to be endemic in Pokot county, in the north-eastern district of Nakapiripirit, in a single focus that spreads into the neighbouring Kenyan district of West Pokot and affects the semi-nomadic pastoralists of the Pokot tribe. Although the VL in this area is known to be caused by Leishmania donovani, with Phlebotomus martini the primary and perhaps sole vector (Wykoff et al., 1969), there are few epidemiological data on the disease in the Pokot. In this endemic focus, the proportion of VL patients co-infected with HIV is believed to be low, as, in an unlinked anonymous survey conducted by Médecins Sans Frontières (MSF) at Amudat Hospital, the main hospital for Nakapiripirit district, only three (1.4%) of the 203 VL patients checked in 2002–2003 were found to be HIV-positive (F. Chappuis, unpubl. obs.). When MSF opened a VL programme in Amudat in 2000, establishing the only treatment centre for VL in the area, meglumine antimoniate (SbV) was used as the first-line drug and AmB as the second-line.

In 2003, however, technical problems led to an interruption in the production of SbV by Aventis. As adequate quantities of Pentostam could not be quickly obtained and the generic form of SSG was not authorized for importation into Uganda, the first-line treatment of VL at Amudat was temporarily switched to AmB. The present article provides a descriptive analysis of this use of AmB for the first-line treatment of VL, focusing on the drug’s safety and effectiveness. This appears to be the first report on the use of AmB against VL in Africa.

PATIENTS AND METHODS

Amudat Hospital, in the town of Amudat, is a 120-bed rural hospital with neither surgical nor radiological facilities. The hospital’s laboratory performs basic tests such as the preparation and checking of thick blood-smears (for malaria), colorimetric haemoglobin estimations (using the Lovibond method), leucocyte counts, stool examinations, urine analyses, Gram staining, sputum examinations, and serology for HIV, hepatitis B, syphilis and brucellosis. Apart from the use of dipsticks for estimating blood concentrations of glucose, no biochemical tests are performed. Since 2001, the diagnosis of VL at the hospital has been based on an algorithm that combines the results of direct agglutination tests (DAT) and, for the suspected cases with ‘borderline’ DAT titres of 1:1600–1:12,800, the microscopical examination of Giemsa-stained spleen aspirates. The DAT diagnostic titres were locally validated, against spleen aspirates, in 2000 and 2001 (F. Chappuis, unpubl. obs.). Demographical
and clinical data, such as age, gender, origin, symptoms on admission, duration of symptoms, weight and height, were collected for every suspected case of VL, as part of the MSF programme’s routine monitoring.

Between September 2003 and April 2004, patients were treated with AmB in its branded form (Fungizone™; Bristol-Myers Squibb, New York, NY) or its generic form (Photericin B; Cipla, Mumbai, India), depending on which drug was available in the country. The decision to use AmB was an operational decision, as no antimonials were available in the country. The drug was administered as a daily dose of 1 mg/kg bodyweight, with each dose given in a slow infusion, over 8–12 h, to improve tolerance. The total dose was 15 mg/kg, given on alternate days over a 30-day period in order to reduce the risk of nephrotoxicity. Renal-function tests could not be performed. If oedema was present, the dose was reduced by 10%, to 0.9 mg/kg. Patients were stimulated to drink abundantly. Access to safe drinking water was secured and oral-rehydration salts were distributed. If clinical dehydration was observed, intravenous rehydration, with 0.9% NaCl or Ringer’s lactate solution, was initiated. Patients were given daily potassium and magnesium supplements, in addition to multi-vitamin, vitamin-C, folic-acid and ferrous-sulphate tablets. All patients received supplementary feeding. Body temperatures were monitored daily and weights weekly. Antibiotic treatments for suspected bacterial infections and blood transfusions for severe anaemia were given, if necessary. Concomitant nephrotoxic medications were avoided.

Initial outcome was assessed, from the patient’s general condition, spleen size, and haemoglobin concentration, on the 25th day of treatment. Initial cure was defined as the clearance of fever plus an improvement in general condition, a decrease in spleen size, and an increase in haemoglobin concentration. If the baseline symptoms persisted and/or there was no reduction in spleen size, a spleen aspirate was collected and checked for amastigotes, as a Giemsa-stained smear. If the aspirate was then found positive, the treatment was prolonged at the same dose until two successive negative spleen aspirates had been obtained (when the patient was defined as a slow responder). Treatment failures included both the non-responders (defined as those who showed neither clinical nor parasitological response to treatment) and the patients who had relapses, with VL diagnosed within 6 months of the initial cure. Definite cure was defined as the absence of relapse 6 months after hospital discharge. Although logistic constraints prevented active follow-up after discharge, most relapse cases would presumably have presented at Amudat Hospital again, as the hospital was the only centre offering treatment for VL in the whole endemic area during the study period. The frequencies of initial cure, in-hospital death and treatment failure were chosen as primary outcomes, whereas the variations in haemoglobin concentration and spleen size, between admission and the end of treatment, were taken as secondary outcomes.

The occurrence of adverse effects was assessed daily, during the clinician’s rounds. In order to minimize any non-reporting bias resulting from the patients (most of whom speak Pokot as a first language) not understanding the Luganda or English used by the clinicians, a weekly questionnaire targeting 19 symptoms was administered, by a translator speaking Pokot, to the sub-group of patients admitted between December 2003 and February 2004. Adverse effects were graded as mild/moderate or, if treatment interruption was necessary, severe. Association of the adverse effects with the drug was classified as possible, probable or certain.

For comparison with the results of the AmB treatment, an historical cohort, of all the patients diagnosed with first-time VL when they presented at the Amudat Hospital between September 2002 and
April 2003, was selected. The patients in this cohort had all been treated with intramuscular injections of SbV, given at 20 mg/kg.day (without an upper limit) for 30 days. The baseline characteristics, outcomes and severe adverse effects among the patients treated with AmB were compared with those of the patients given SbV.

Data were entered in an Excel (Microsoft) spreadsheet by the clinician in charge. The database was subsequently cleaned by identifying aberrant data. Categorization of nutritional status was based either on weight-for-height, for girls who were <137 cm tall and boys who were <145 cm tall, or on body mass indexes (BMI). Weights-for-height that were <70%, 70%–79%, 80%–89% and >89% of ‘normal’ (Anon. 1977) or BMI of <16, 16–17, >17–18, and >18 were taken as indications of severe, moderate, mild, and no malnutrition, respectively. The statistical analyses were performed using the STATA TM software package (StataCorp, College Station, TX). Between-cohort comparisons were made using one-way analyses of variance for the continuous variables, Kruskal–Wallis tests for the discrete variables (the estimates of haemoglobin concentration), and χ² or Fisher’s exact tests for the proportions. Results are presented with 95% confidence intervals (CI), where appropriate. A P-value of <0.05 was considered indicative of a statistically significant difference.

RESULTS

Demographic and clinical characteristics of the 210 patients treated with AmB are shown in Table 1. The presenting symptoms most frequently reported by the patients were abdominal mass (96.2%), fever (92.8%), cough (68.9%), headache (39.7%), weight loss (31.6%), anorexia

| TABLE 1. The demographic and clinical characteristics, on admission, of 371 patients with visceral leishmaniasis who were treated with amphotericin B deoxycholate (AmB) or meglumine antimoniate (SbV) at Amudat Hospital, in north–eastern Uganda |
|-------------------------------------------------|-----------------|-----------------|
| NO. AND (%) OF PATIENTS:                        | AmB cohort      | SbV cohort      |
| Evaluated                                       | 210             | 161             |
| Aged (years)                                    |                 |                 |
| 0–5                                             | 42 (20.0)       | 32 (19.9)       |
| >5–15                                           | 101 (48.1)      | 71 (44.1)       |
| >15                                             | 67 (31.9)       | 58 (36.0)       |
| Male                                            | 154 (73.3)      | 116 (72.0)      |
| Female                                          | 56 (26.7)       | 45 (28.0)       |
| From Uganda                                     | 59 (28.1)       | 44 (27.3)       |
| From Kenya                                      | 151 (71.9)      | 117 (72.7)      |
| With no or mild malnutrition                    | 92 (44.7)       | 69 (43.7)       |
| With moderate malnutrition                      | 61 (29.6)       | 38 (24.0)       |
| With severe malnutrition                        | 53 (25.3)       | 51 (32.3)       |
| With anaemia that was                           |                 |                 |
| Severe (<5 g haemoglobin/dl)                    | 3 (1.4)         | 4 (2.5)         |
| Moderate (5–7 g haemoglobin/dl)                 | 60 (28.7)       | 75 (46.6)       |
| Mild (>7–11 g haemoglobin/dl)                   | 139 (66.5)      | 82 (50.9)       |
| Without anaemia (>11 g haemoglobin/dl)         | 7 (3.3)         | 0 (0.0)         |
| Mean (s.d.) spleen size (cm)                    | 13.7 (4.2)      | 13.3 (4.1)      |
| Median duration of illness and (interquartile range) (weeks) | 4 (4–12)        | 7.7 (4–8)       |

*On admission, the two cohorts were similar in terms of all the recorded characteristics (P>0.2) apart from anaemia; moderate–severe anaemia was significantly more common in the patients given SbV (P<0.001).
(21.5%), epistaxis (14.3%), weakness (9.1%), oedema (5.3%) and vomiting (2.9%). The diagnosis of VL was based only on a DAT titre of at least 1:25,600 (181 cases) or on an amastigote-positive spleen aspirate (29 patients). Only branded AmB was given to 114 (54.3%) patients whereas 74 patients (35.2%) were treated only with generic AmB and 22 (10.5%) received both products. Twenty-five (11.9%) patients with malaria, 22 (10.5%) with respiratory tract infections, and 21 (10.0%) with other bacterial infections (such as typhoid fever, otitis media, pharyngitis, brucellosis or dysentery) were given specific treatments for their accompanying infections.

Outcome of AmB Treatment
Of the 210 patients treated with AmB, 194, including one slow responder, were initially cured, resulting in an initial cure ‘rate’ of 92.4% (CI=87.9%–95.6%). Ten of the patients died during their stay in the hospital, giving a case-fatality ‘rate’ of 4.8% (CI=2.4%–8.8%). The causes of the 10 in-hospital deaths were bleeding (three patients), infectious complications (two), severe anaemia (one), cardiac failure (one), ileus (one) or unknown (two). Six patients relapsed, resulting in a treatment failure ‘rate’ of 2.9% (CI=1.2%–6.4%). By the end of treatment, compared with their baseline values, the median haemoglobin concentration had increased by 1.4 g/dl and the mean spleen size had fallen by 5.3 cm. The case-fatality ‘rates’ among the patients treated with just branded AmB (5.3%), just generic AmB (5.4%), or a combination of the two products (0.0%) were not significantly different ($P>0.20$). The patients given only the branded drug were more likely to fail treatment than the patients given only the generic drug but the difference did not quite reach statistical significance (4.4% v. 0.0%; $P=0.08$).

Tolerance of AmB Treatment
The mean total dose of AmB administered was 13.6 mg/kg, corresponding to a mean daily dose of 0.93 mg/kg. Adverse effects requiring interruption of treatment — vomiting (two patients), itching (one) or an anaphylactic reaction (one) — occurred in four patients (1.9%; CI=0.5%–4.8%). All four patients recovered. Adverse effects in 55 consecutive patients (26% of those treated with AmB) were recorded on the standardized questionnaire by an interviewer speaking the patients’ first language. The main reported adverse effects in this sub-group were fever (52.7%), sweating (40%), abdominal pain (38.9%), headache (37.0%), diarrhoea (22.2%), itching (14.8%), and/or shivering (14.5%). All these adverse effects were considered as mild to moderate, and none required an interruption of treatment. The association of these adverse effects with the treatment was considered as probable in 54% of cases, possible in 43%, and unknown in 3%. The intensity of all the adverse effects recorded decreased during the course of the treatment. Only 29% of the 55 patients in the sub-group did not mention any adverse effects.

Comparison with the Patients Treated with SbV
The demographic characteristics of the cohort of 210 patients treated with AmB were similar to those of the historical cohort of 161 patients treated with SbV (see Table 1). The clinical characteristics on presentation were also similar, with the exception that a higher proportion of patients in the SbV group presented with moderate–severe anaemia, 49.1% of the patients given SbV but 30.1% of those given AmB having $<7$ g haemoglobin/dl ($P<0.001$). The frequencies of initial cure, in-hospital death and treatment failure were also similar in the two cohorts (Table 2). One patient did not respond to SbV and was switched to AmB, with a good clinical response. The treatment-attributable reduction in spleen size was significantly greater with SbV than with AmB (6.8 v. 5.3 cm;
Although the median increase in haemoglobin concentration was also greater with SbV than with AmB (2.4 vs. 1.4 g/dl; \( P = 0.032 \)), the mean haemoglobin concentration recorded at the end of SbV treatment was identical to that recorded at the end of the AmB treatment (9.7 g/dl; \( P = 0.13 \)). The only adverse effect that required interruption of SbV treatment was clinical pancreatitis, which occurred in two patients. Thus, only 1.2\% (CI = 0.1\%–4.4\%) of the patients given SbV and 1.9\% (CI = 0.5\%–4.8\%) of those given AmB developed adverse effects that required the interruption of treatment (\( P > 0.20 \)).

**DISCUSSION**

For the treatment of VL in Amudat, Uganda, amphotericin B deoxycholate, either in its branded or generic form, appears as effective and safe as meglumine antimoniate. The case-fatality 'rate' seen among the AmB-treated patients in the present study (4.8\%) was slightly higher than those recorded in India (Sundar et al., 2004; Thakur and Narayan, 2004). In the trials of AmB among Indian cases of VL, however, strict exclusion criteria (such as any serious concurrent infection, neutropenia or severe anaemia) led to the exclusion of the most severe cases (Sundar et al., 2004; Thakur and Narayan, 2004). In Africa, similar or higher case-fatality rates to those seen with AmB in Uganda (present study) have been reported among VL cases given other treatments (Veeken et al., 2000; Moore et al., 2001; Ritmeijer et al., 2001, 2006). In the present study, the frequency of severe adverse effects requiring the interruption of AmB treatment was low (1.9\%), and an active search for adverse effects, by someone speaking the patients' first language, revealed only mild or moderate adverse-effects that decreased in severity during treatment.

In terms of clinical outcome and the incidence of severe adverse effects, the cohort treated with AmB was very similar to the historical cohort of patients treated with SbV. The statistical comparison of these two cohorts does, however, have some limitations. Firstly, the two cohorts are consecutive and not concurrent. In the absence of randomization, discrepancies in the demographic or clinical characteristics of the patients might have remained unnoticed. Secondly, the physicians who assessed the patients changed during the study period, leading to possible between-cohort differences in the general management of the patients, the manual evaluation of spleen size, and/or the reporting of adverse effects.

The low frequency of failure (2.9\%) observed in the present study indicates that AmB can be considered an effective drug for the treatment of VL in Uganda. As secondary resistance to AmB is believed to be an unlikely phenomenon in the absence of HIV co-infection (Bryceson, 2001), the few treatment failures that did occur are unlikely to be the result of such resistance in the local *L. donovani*. There are some indications that, to be effective, AmBisome needs to be given in higher doses in Africa than in India.

### TABLE 2. Clinical outcomes of the 371 patients with visceral leishmaniasis who were treated with amphotericin B deoxycholate (AmB) or meglumine antimoniate (SbV) at Amudat Hospital, in north–eastern Uganda

<table>
<thead>
<tr>
<th>Outcome</th>
<th>AmB cohort (No. and [%])</th>
<th>SbV cohort (No. and [%])</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial cure</td>
<td>194 (92.4) [87.9%–95.6%]</td>
<td>153 (95.0) [90.4%–97.8%]</td>
<td>&gt;0.20</td>
</tr>
<tr>
<td>Death</td>
<td>10 (4.8) [2.4%–8.8%]</td>
<td>6 (3.7) [1.5%–8.3%]</td>
<td>&gt;0.20</td>
</tr>
<tr>
<td>Treatment failure</td>
<td>6 (2.9) [1.2%–6.4%]</td>
<td>2 (1.2) [0.2%–4.9%]</td>
<td>&gt;0.20</td>
</tr>
</tbody>
</table>
(Berman et al., 1998). This might also be true for AmB, although the rarity of treatment failure in the present study indicates otherwise. As the present patients were not actively followed up after hospital discharge, the possibility that there were unrecorded cases of relapse remains. The number of such missed relapses was almost certainly small, however, given that Amudat Hospital was the only functional VL-treatment centre in the area during the study period. Although the frequency of treatment failure among the patients given Sb\textsuperscript{V} was very similar to that among the patients given AmB, the reductions in spleen size and the increases in haemoglobin concentration during treatment were more marked in the Sb\textsuperscript{V} group. The latter finding should be interpreted with caution, however, since the two cohorts differed in their mean haemoglobin concentrations on admission (Table 1).

The relatively good tolerance of AmB observed in the present study is probably related to the long infusion time, the maintenance of adequate hydration and the supplementation of electrolytes. There is some evidence that a relatively slow infusion of AmB (4 h v. 45 min) reduces the occurrence of infusion-related adverse effects (Ellis et al., 1992). Although the administration of AmB by continuous infusion over 24 h appears to reduce nephrotoxicity (Eriksson et al., 2001), such long infusions were not feasible at Amudat Hospital. As it was also not possible to monitor renal function at the study hospital, AmB was given on alternate days, although there is no clear evidence that this reduces the risk of nephrotoxicity (Thakur et al., 1994). The toxicity of AmB appears to vary with the patient population, the underlying clinical condition of the patients, the use of concomitant nephrotoxic drugs, and the mode of administration (Girmenia et al., 2001; Mayer et al., 1999). Fortunately, acute renal failure appears to be rare among VL patients given the drug (Thakur et al., 1999), although transient increases in creatinine concentrations are observed in up to 20% of such patients (Thakur et al., 1994). AmB should be considered as a relatively safe drug in VL patients, provided that proper attention is given to the practical aspects of drug administration, including the permanent presence of nursing staff to monitor infusion flow. The workload of the medical and nursing team involved is clearly greater with slow infusions of AmB than with intramuscular injections of antimonial drugs given once daily. Moreover, the use of AmB is logistically more demanding, as it requires a cold chain and significant capacities for the transport and storage of intravenous fluids.

In conclusion, conventional AmB is an effective and reasonably safe drug for the treatment of VL in Uganda. Liposomal AmB would probably be even better tolerated and a more practical option, reducing the duration of the hospital stay (Bern et al., 2006). A recent study conducted in Ethiopia showed that miltefosine could be an interesting therapeutic alternative for the treatment of VL in East Africa (Ritmeijer et al., 2006). Moreover, in clinical trials co-ordinated by the Drugs for Neglected Diseases initiative, the potential usefulness of paromomycin in the treatment of VL is currently being evaluated in several East African countries. The number of treatment options for VL, preferably using drugs in combination (Bryceson, 2001), is therefore likely to increase in the coming years. In the mean time, the main priority in sub-Saharan Africa, where resistance to antimonials is still a negligible problem, is to ensure affordable and sustainable access to antimonials for the neglected populations suffering from VL (Berman et al., 1998; Yamey and Torreele, 2002).

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REFERENCES


study of 938 cases. Transactions of the Royal Society of Tropical Medicine and Hygiene, 93, 319–323.