The use of monoclonal antibody-based ELISAs to monitor the efficacy of drugs against male Onchocerca gibsoni in vitro

S. J. More, D. B. Copeman

Graduate School of Tropical Veterinary Science and Agriculture, James Cook University of North Queensland, Townsville, Australia

Abstract

Four monoclonal antibodies directed against antigens of *Onchocerca gibsoni* were used in antigen detection ELISAs to monitor the efficacy of CGP 20309, CGP 20376, CGP 21833, CGP 24589 and CGP 26702 at 5 µg/ml against male *O. gibsoni* in vitro. No significant differences ($P < 0.05$) in antigen output between treated and control groups of parasites were recorded. However, consistently higher levels of antigen from treated (CGP 21833) as compared to control parasites were measured with all four assays, with differences being higher in the first 2 to 3 days post treatment than subsequently. The sensitivity of comparisons between groups was reduced by the high variability in output of antigen both between worms and also from the same worm, in part as a result of mechanical damage to worms sustained during collection or manipulation in vitro. This problem was reduced by zero handling once worms were established in vitro and it is recommended that future work should include a 24 to 48 hour period before treatment commences to detect raised antigen levels associated with physically damaged parasites so they can be excluded. It was concluded that this type of assay has no intrinsic technical or logistical advantage over other published methods of assessing drug-related damage in in vitro filarial screens. Nevertheless, further work using antigen detection ELISAs in this context is justified since these assays, unlike all other methods of assessing drug-induced damage in vitro, have direct application for use in identifying chemotherapeutic effects against similar parasites in vivo.

Introduction

In vitro screens using a variety of nematodes have been utilised in the search for safe effective chemotherapeutic agents against *Onchocerca volvulus*. All shared a common problem; that of determining the extent to which treated worms were damaged by the drug. Most techniques were based on visual or measured assessment of motility (Nowak et al., 1987; Pax et al., 1988; Satti et al., 1988) but a variety of biochemical tests sensitive to the metabolic functions of the parasite were also developed (Comley et al., 1988; Comley and Rees, 1989).

Another different method is described in this study. It relies on the possibility that there may be a change in the nature or amount of antigenic material released into the medium after exposure of cultured male *Onchocerca gibsoni* to filaricidal compounds, and that detection of such antigens with monoclonal antibody-based ELISAs may be a means by which drug-induced damage could be recognised. Furthermore, there is the additional possibility that such in vitro tests could be used as a primary screen of antigen detection ELISAs; promising assays then being applied to serum or urine to monitor the effectiveness of the same compounds in cattle against *O. gibsoni* and in man against *O. volvulus*.

Materials and methods

Isolation and in vitro maintenance of male O. gibsoni

Male *O. gibsoni* were dissected from nodules freshly collected from a local abattoir before placing them individually in 1.5 ml of DMEM containing antibiotics (120 mg/l benzylpenicillin, 200 mg/l streptomycin sulphate) for 2 h. Motile parasites with no visible evidence of damage were then maintained at 37 °C in separate wells of a 24 well plate each containing 2 ml of DMEM with 20 mM Hepes [(N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulphonic acid]); Boehringer Mannheim GmbH, Mannheim, FRG], 60 mg/l benzylpenicillin and 100 mg/l streptomycin sulphate.

In vitro trials

Trial 1

A total of 57 male *O. gibsoni* were maintained individually in medium without drugs for 5 days. At 24 h intervals each parasite was carefully transferred to 2 ml of fresh medium and the spent supernatant fluid was collected and stored at −70 °C for later measurement of detectable antigen. Eight parasites were withdrawn during the trial because of visible cuticular damage.

Trial 2

A total of 105 male *O. gibsoni* were maintained individually in vitro for a period of 3 days at 37 °C, without a change in medium, to investigate the effect of drug therapy on the levels of detectable parasite antigen released into the supernatant fluid. Five macrofilaricidal drugs (CGP 20309, CGP 20376, CGP 21833, CGP 24589 and CGP 26702; kindly supplied in pure form by H. P. Striebel and J. J. Gallay, Ciba Geigy Pharma International, Basel, Switzerland) were used at a final concentration of 5 µg/ml, each with a replicate of 10 par-

Accepted 26 November 1990

Trop. Med. Parasitol. 42 (1991) 21–24

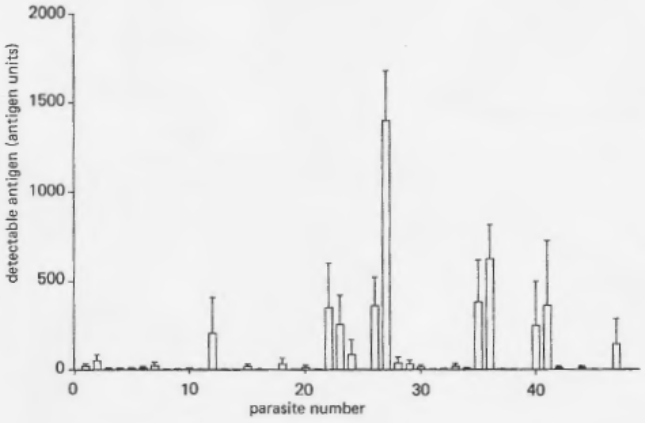

Fig. 1 The mean (±SE) daily output of antigen from 49 untreated male *Onchocerca gibsoni* in vitro measured using the Og5A4-based ELISA. In this figure, no account has been made for the significant difference that was measured between antigen levels on subsequent days. One antigen unit is defined as the amount of the target antigen of Og5A4 found in 1 ng of a crude extract of male *Onchocerca gibsoni*

asites; in association with untreated (n = 45) and solvent (10 µl DMSO/2 ml of medium; n = 10) controls. Drugged medium, which was made freshly at the beginning of the trial, contained 10 µl of a 1 mg stock solution of each drug (that is, 1 mg of drug/ml of DMSO) per 2 ml of DMEM/20 mM Hepes to give a final concentration of 13.2 µM CGP 20309, 13.0 µM CGP 20376, 13.8 µM CGP 21833, 12.3 µM CGP 24589 and 12.8 µM CGP 26702. The supernatant fluid was collected at the end of 3 days and stored at −70 °C for later use.

Trial 3

Parasites in an untreated (n = 10) and treated [5 µg/ml (13.8 µM) CGP 21833; n = 10] group were maintained for 7 d in vitro. At 12 h, and then daily, 1 ml of spent supernatant fluid was removed for storage at −70 °C for later analysis and replaced with 1 ml of fresh medium. Medium containing CGP 21833 was prepared daily using the method described in Trial 2. One parasite in the treatment group was withdrawn during the trial with visible evidence of physical damage, and results from one randomly chosen untreated control parasite were also removed prior to statistical analysis.

Antigen detection ELISAs

The antigen detection ELISAs used in this study have been previously described, based on the monoclonal antibodies Og4C3 (More and Copeman, 1990), Og5A4, Og5A6 and Og5C3 (More and Copeman, 1991).

Statistical analyses

Analysis of variance, student t-test and Tukey's pairwise comparison of means test were used to compare differences in antigen levels between control and treated groups of parasites. In most cases data were transformed [$\log_{10}(x+1)$] for analysis.

Results

The daily output of antigen released from male *O. gibsoni* in trial 1 was highly variable between worms ($P < 0.005$) as may be seen in the example illustrated in Fig. 1. In addition this figure demonstrates that daily antigen output of some worms also varied greatly, especially those which yielded high levels of antigen. It was concluded on the basis of microscopic examination that such high yields were probably the result of mechanical damage to worms sustained during collection or manipulation in vitro as large increases in antigen output preceded stereoscopic evidence of physical damage by some days.

The mean daily output of antigen from parasites in trial 1 also varied significantly over time ($P < 0.005$) when measured with the Og5A4 and Og5A6-based ELISAs and the trend was both linear and decreasing. Significant differences in antigen output over time were also measured in trial 3 with the assays based on Og4C3 ($P = 0.011$) and Og5A6 ($P = 0.043$); in contrast the output of antigen measured with assays based on Og5A4 and Og5C3 remained constant over time (Fig. 2).

There was no significant difference in output of antigen between the various drug treatment groups or between control and treatment groups in trial 2 for any of the assays, except that based on Og5A4 where antigen levels from worms treated with CGP 26702 were higher than those from worms treated with CGP 20376. However, in trial 3 (see Fig. 2) there was evidence of higher levels of antigen released from treated (CGP 21833) than from control groups when measured with the assays based on Og4C3 ($P = 0.098$), Og5A4 ($P = 0.107$), Og5A6 ($P = 0.138$) and Og5C3 ($P = 0.082$). Furthermore, using all four assays, the differences in antigen output between the treated and control groups tended to the higher during the first 3 as compared to the last 4 days of the trial.

In an extension of this trial (More and Copeman, unpublished), significant differences between the viability of parasites in treated and control groups were measured using MTT-formazan colorimetry, lactate output and motility. The treated parasites were essentially immotile within 6 hours post treatment. Using the biochemical criteria, significant differences between treated and control groups of parasites were detected 24 hours after treatment commenced, with a continuing decline in viability followed by death 3 to 4 days later.

Discussion

The concentration of drugs used in this study (5 µg/ml) was chosen to mimic maximum plasma values achieved in humans and chimpanzees following treatment with therapeutic doses of CGP 20376 and CGP 6140 (Lecail-

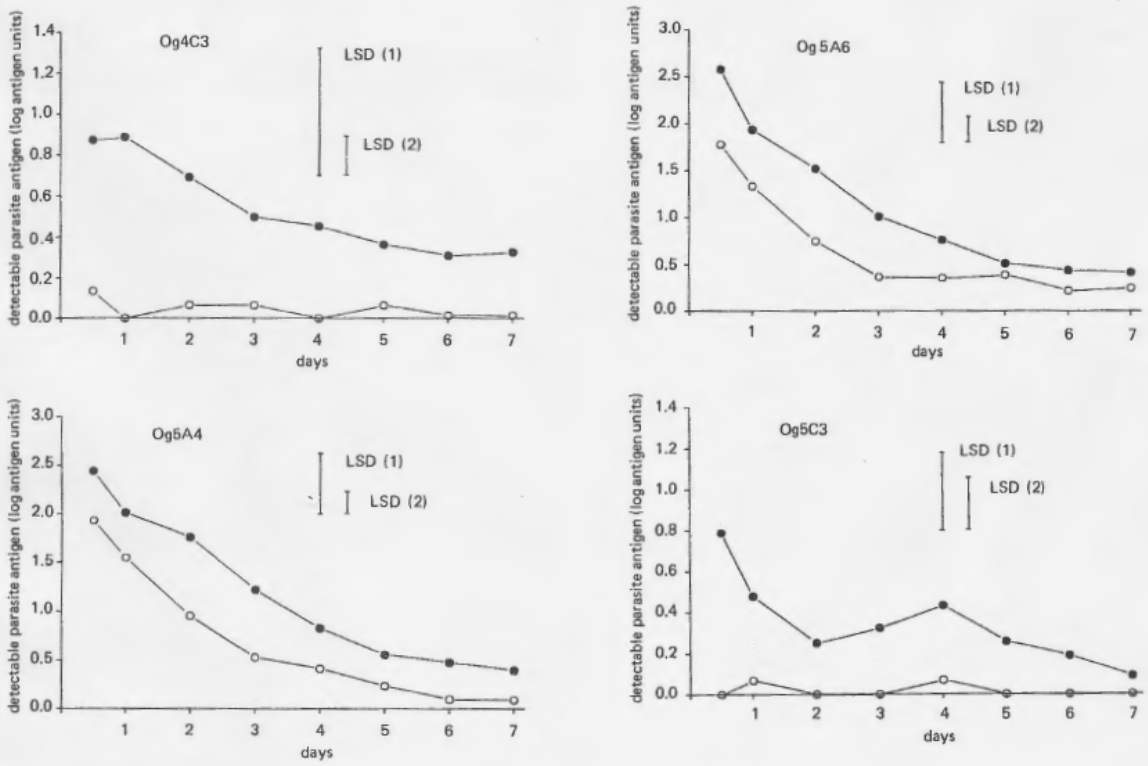

Fig. 2 The mean (±SE) output of antigen from untreated (○) or CGP 21833-treated (●) male *Onchocerca gibsoni* in vitro measured using the 4 antigen detection ELISAs. The least significant difference ($P = 0.05$) of the mean levels of measured antigen between the treatment and control groups [LSD (1)], and at different times [LSD (2)] is presented on each figure. The results shown are the actual antigen levels measured on consecutive days with no allowance made for the removal and replacement of 50% of the medium every 24 hours. One antigen unit is defined as the amount of the target antigen of each monoclonal antibody found in 1 ng of a crude extract of male *Onchocerca gibsoni*

lon et al., 1987; Moysan et al., 1988; Strote, 1989). Furthermore, most recent in vitro chemotherapeutic research with *Onchocerca* spp. has utilised CGP compounds at drug concentrations similar to those described in this paper (Strote, 1987; Townson et al., 1987; Pax et al., 1988).

Conclusions with may be drawn about the usefulness of antigen detection immunoassays and the concept of using such assays for monitoring efficacy of drugs against *O. gibsoni* in vitro are clouded by the large variation which occurred in output of antigen both between worms and also from the same worm in this study, and the consequent reduction in sensitivity of measuring differences between treatment and control groups.

It became apparent as the work progressed that worms with the largest variation in antigen output were those that had sustained physical damage from handling procedures. Indeed, the assays were highly sensitive indicators of iatrogenic damage, with large increases in antigen output preceding stereoscopic evidence of physical damage by some days.

In an attempt to reduce the physical damage to worms which occurred in trial 1 when worms were transferred daily to new medium, a policy of zero handling was adopted in trials 2 and 3 once worms were established in vitro. Furthermore, even greater care was taken in trial 3 to examine and exclude damaged worms at the outset than in trials 1 and 2. The outcome was less within worm variability in trial 3 than in trials 1 and 2. As a consequence there was evidence of differences in output of antigen by worms treated with CGP 21833 compared with controls with all assays in trial 3; whereas no significant differences were found in trial 2 between the various treatment and control groups.

It thus seems likely that even more effective steps to exclude physically damaged worms may make this method of monitoring drug-induced damage more sensitive and therefore more useful that current results portray. To this end it is recommended that, in future trials, the output of antigen from individual worms should be monitored for 48 hours before commencement of treatment. These data could then be used to identify and eliminate parasites subtly damaged during their recovery from nodules as well as provide a pretreatment antigen level for comparison with levels post treatment. The use of tests such as the repeated measures analysis of variance

could then provide a powerful method of identifying possible differences in antigen output induced by the effects of drugs.

Since worms were paralysed from about 6 hours after being immersed in drugged medium and dead (as measured by MTT-formazan colorimetry and lactate output) after about the third day, it appears that antigens detected by the monoclonal antibodies used in these assays were unassociated with movement and not actively excreted or secreted, as antigen was still emanating from some worms 7 days after treatment commenced. Nevertheless, it may be observed in Fig. 2 that differences in output of antigen between treated and control groups tended to be higher in the first 3 than the last 4 days of trial 3 for all assays, suggesting that at least some of the epitopes recognised may have been metabolic products. This result also demonstrates that future trials need not proceed for more than 3 days to produce a useful result.

As a technique, the use of antigen detection ELISAs based on monoclonal antibodies to monitor effects of drugs on *O. gibsoni* in vitro has no obvious technical or logistical advantages over other methods which have been used, such as MTT-formazan colorimetry, lactate output or measurement of parasite motility (Nowak et al., 1987; Comley et al., 1988). The further development of such monoclonal antibody-based assays cannot, therefore, be promoted on these grounds alone. However, such ELISAs may be useful as a means of predicting filaricidal damage to *O. gibsoni* (and *O. volvulus*) in vivo (More and Copeman, 1991), an application which is not possible with any other method currently used to assess filarial viability in vitro. In this context, further work with antigen detection ELISAs in the male *O. gibsoni* in vitro model is justified. It must be remembered, however, that this procedure would be unlikely to identify suitable assays when used in conjunction with drugs which are effective only in unison with host effector mechanisms or following metabolism in vivo.

Acknowledgements

We wish to thank R. M. Patterson and W. L. Howell for supplying the hybridomas from which these monoclonal antibodies were selected and G. De'ath for assistance with the statistical calculations (all from the Graduate School of Tropical Veterinary Science and Agriculture, James Cook University of North Queensland, Townsville, 4811). The work was funded, in part, by the Edna McConnell Clark Foundation.

References

Comley, J. C. W., M. J. Rees: Radiorespirometric detection of macrofilaricidal activity in vitro. Parasitology 98 (1989) 259–264

Comley, J. C. W., M. J. Rees, A. B. O'Dowd: The application of biochemical criteria to the assessment of macrofilarial viability. Trop. Med. Parasitol. 39 (1988) 456–459

Lecaillon, J. B., A. Sioufi, C. Souppart, J. P. Dubois: Determination of CGP 6140 and its N-oxide metabolite, CGP 13 231, in plasma and urine by columns or cartridge switching HPLC. Chromatographia 24 (1987) 876–880

More, S. J., D. B. Copeman: A highly specific and sensitive monoclonal antibody-based ELISA for the detection of circulating antigen in bancroftian filariasis. Trop. Med. Parasitol. 41 (1990) 403–406

More, S. J., D. B. Copeman: The use of monoclonal antibody-based ELISAs to monitor hemotherapeutic effects in the bovine-*Onchocerca gibsoni* drug screen. Trop. Med. Parasitol. 42 (1991) 17–20

Moysan, F., M. van Hoegaerden, R. W. Cooper, S. C. Bhatia, A. A. Poltera, H. P. Striebel, B. Ivanoff: Antifilarial activity of CGP 20 376 in chimpanzees (*Pan t. troglodytes*) naturally infected with *Dipetalonema vanhoofi*. Trop. Med. Parasitol. 39 (1988) 35–39

Nowak, M., G. W. Hutchinson, D. B. Copeman: In vitro drug screening in isolated male *Onchocerca gibsoni* using motility suppression. Trop. Med. Parasitol. 38 (1987) 128–130

Pax, R. A., J. F. Williams, R. H. Guderian: In vitro motility of isolated adults and segments of *Onchocerca volvulus*, *Brugia pahangi* and *Acanthochellonema viteae*. Trop. Med. Parasitol. 39 (1988) Suppl., 450–455

Satti, M. Z., E. A. VandeWaa, J. L. Bennett, J. F. Williams, G. A. Conder, J. W. McCall: Comparative effects of anthelmintics on motility in vitro of *Onchocerca gutturosa*, *Brugia pahangi* and *Acanthocheilonema viteae*. Trop. Med. Parasitol. 39 (1988) 480–483

Strote, G.: In vitro study on the effect of the new chemotherapeutic agents CGP 6140 and CGP 20376 on the microfilariae of *Onchocerca volvulus*. Trop. Med. Parasitol. 38 (1987) 211–213

Strote, G.: Studies on the activity of the Ciba Geigy compounds CGP 6140, 20376, 20309 and 21833 against third and fourth stage larvae of *Onchocerca volvulus*. Trop. Med. Parasitol. 40 (1989) 51–56

Townson, S., C. Connelly, A. Dobinson, R. Muller: Drug activity against *Onchocerca gutturosa* males in vitro: a model for chemotherapeutic research on onchocerciasis. J. Helminth. 61 (1987) 271–281

Dr. Simon More

Graduate School of Tropical Veterinary Science and Agriculture
James Cook University of North Queensland
Townsville Q 4811, Australia