The use of monoclonal antibody-based ELISAs to monitor chemotherapeutic effects in the bovine-Onchocerca gibsoni drug screen

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Abstract

Three monoclonal antibodies directed towards antigens of *Onchocerca gibsoni* were used in antigen detection ELISAs to detect parasite antigens in sera from 100 cattle infected with *O. gibsoni*, in trials with the filaricidal compounds CGP 6140, CGP 20309, CGP 20376, CGP 21833, CGP 24589 and CGP 26702. Measurable levels of parasite antigens were highly variable, both within and between treatment and control groups of animals, with no consistent trends which related to time after treatment, micro or macrofilaricidal effects against *O. gibsoni*, or dose rate for any of the compounds used. It was concluded that these assays were unsuitable as a method of identifying drug-induced damage to *O. gibsoni* following the administration of these compounds. A detailed protocol for selecting suitable assays is discussed.

Introduction

An urgent need continues for a safe and effective macrofilaricidal drug to treat human onchocerciasis. Putative filaricidal drugs are presently being tested in a number of in vitro and animal screening models, including the bovine *Onchocerca gibsoni* screen (Copeman, 1979).

Assessment of drug effectiveness in cattle against *O. gibsoni* is time-consuming as it involves ante mortem monitoring of dermal microfilariae and post mortem evaluation of drug-related changes to the parasite using histological (Striebel, 1988) and embryogram (Schulz-Key et al., 1980; Vankan and Copeman, 1988) techniques. In an effort to accelerate this process, a number of researchers have applied immunological methods to the assessment of drug efficacy. Studies have been based on the postulate that the level or pattern of release of specific parasite antigens in host serum could be monitored following chemotherapy, with evidence of drug effectiveness provided by a measured alteration in antigen levels. This concept has been shown to be feasible by a number of workers using a range of filarial species and a number of immunological methods of detecting parasite antigens (Carme et al., 1982; Forsyth et al., 1984; Malhotra and Harinath, 1984; Weil et al., 1986; Vankan et al. 1987; Weil et al., 1988).

This paper describes the application of monoclonal antibody-based antigen detection enzyme-linked immunosorbent assays (ELISAs) as a means of assessing drug efficacy in the bovine-*O. gibsoni* drug screen. The ELISAs were used to monitor specific parasite antigen levels in the serum of cattle naturally infected with *O. gibsoni*, in trials with known filaricidal drugs.

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Materials and methods

Bovine-O. gibsoni screen

A total of 100 Brahman cross cattle about 2.5 years old were selected for this study because they had high (> 100) numbers of microfilariae in 8 mm diameter cores of midline ventral thoracic skin [as described by Forsyth et al. (1984) based on methods of Copeman (1979)]. Animals were stratified into treatment groups according to dermal microfilarial count at the beginning of the experiment. All animals were bled before chemotherapy commenced and re-bled at intervals of 1–4 weeks for 3 months. Serum was stored at −70 °C until used.

Six different putative filaricidal drugs (manufactured and supplied by Ciba Geigy Pharma International, Basel, Switzerland) were tested at a number of different dose rates (shown in Table 1). CGP 6140 was supplied as a powder in pure form, the other compounds were supplied as a powder containing 25 % (w/w) active ingredient with the balance being wettable powder (mainly sucrose).

Monoclonal antibodies

Three monoclonal antibodies, directed against *O. gibsoni*, were used during this investigation. Og5A4, an IgM molecule, was directed against phosphorylcholine. Og5A6, an IgG_1, molecule, recognised antigens of both filarial (*O. gibsoni* and *O. volvulus*) and non-filarial (*Ancylostoma caninum* and *Toxocara canis*) nematodes but not phosphorylcholine. The target antigens of both Og5A4 and Og5A6, visualised on histological sections of *O. gibsoni* nodules using the immunoperoxidase technique, are located on many internal structures of this parasite, including the gut, the reproductive tract and the inner membrane of the hypodermis. A further IgG_1 molecule, Og5C3, was directed against a sperm-associated protein of *O. gibsoni*.

Antigen detection ELISAs

Antigen detection ELISAs based on the monoclonal antibodies Og5A4, Og5A6 and Og5C3 were performed as previously described (More and Copeman, 1990). The capturing antibody of the Og5A4-based ELISA was coated at 2 µg of protein/ml of ascitic fluid

Table 1 Protocols used for screening compounds against *Onchocerca gibsoni* in cattle. CGP 6140 was administered subcutaneously, all other compounds were solubilised in water and administered per os using a stomach tube

Drug	Treatment protocol	Total dose (mg/kg)	No. of cattle treated
CGP 6140	50 mg/kg once*	50	3
CGP 20309	10 mg/kg (each 12 h × 3)	30	3
	20 mg/kg once	20	3
	20 mg/kg (each 12 h × 3)	60	3
	40 mg/kg once	40	3
	40 mg/kg (daily × 3)	120	6
	60 mg/kg once	60	3
CGP 20376	5 mg/kg (each 12 h × 3)	15	3
	10 mg/kg once	10	3
	10 mg/kg (each 12 h × 3)	30	3
	20 mg/kg once*	20	3
	40 mg/kg once*	40	3
	60 mg/kg once*	60	6
CGP 21833	40 mg/kg once	40	3
	60 mg/kg once	60	3
CGP 24589	10 mg/kg (each 12 h × 3)	30	3
	20 mg/kg once	20	3
	20 mg/kg (each 12 h × 3)	60	3
	40 mg/kg once	40	3
	40 mg/kg (daily × 3)	120	3
CGP 26702	10 mg/kg (each 12 h × 3)	30	3
	20 mg/kg once	20	3
	20 mg/kg (each 12 h × 3)	60	3
	40 mg/kg once	40	3
	40 mg/kg (daily × 3)	120	3

*solubilised using 10% (w/v) polyethylene glycol (PEG) 200 or PEG 400 to a total volume of 190 ml per animal

*prepared in water containing 5% (v/v) acetic acid; immediately after dosing a further 4 litres of 5% acetic acid was administered per os

precipitated using saturated ammonium sulphate, and the Og5A6- and Og5C3-based assays at 5 and 1 µg of protein/ml, respectively, of Protein A-purified supernatant fluid. Before assaying with the Og5A4- and Og5A6-based ELISAs, the bovine serum was pretreated with heat [as described by More and Copeman (1990) with the modification that equal volumes of serum and 0.1 M Na_2EDTA pH 4.0 were mixed before boiling], but was not preheated before assaying with Og5C3. The term antigen unit (A.U.) was introduced because the proportion of the target antigen of each monoclonal antibody in relation to the total number of antigens present in a whole parasite was unknown. A sample containing 100 A.U. is defined as the amount of the target antigen of each monoclonal antibody found in 100 ng of a deoxycholate-soluble male *O. gibsoni* preparation.

Non-immunological assessment of filaricidal action

Microfilarial numbers in the skin were determined using the method of Copeman (1979). Embryograms were performed as described by Vankan and Copeman (1988) based on methods of Schulz-Key et al. (1980) and the guidelines for histological assessment of drug-related changes to *O. gibsoni* were followed (Striebel, 1988).

Results

Figures 1, 2 and 3 illustrate the levels of parasite antigen detected in serum from cattle infected with *O. gibsoni* and treated or not with CGP 20376, using ELISAs based on Og5A4, Og5A6 and Og5C3, respectively. These figures were selected because they illustrate features of the results obtained with all compounds. There was no consistent trend in levels of detectable antigen that can be ascribed to time after treatment, damage to microfilariae or adults of *O. gibsoni*, or dose rate for any of the compounds used. Due to the small number of animals in each treatment group and the extreme variability within

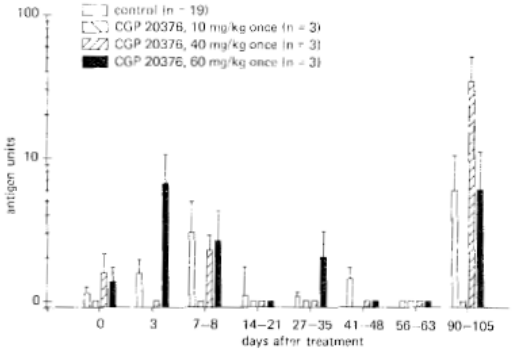

Fig. 1 Levels (mean ± SE) of parasite antigen detected using the Og5A4-based ELISA in serum from groups of *Onchocerca gibsoni*-infected cattle either untreated or treated with CGP 20376

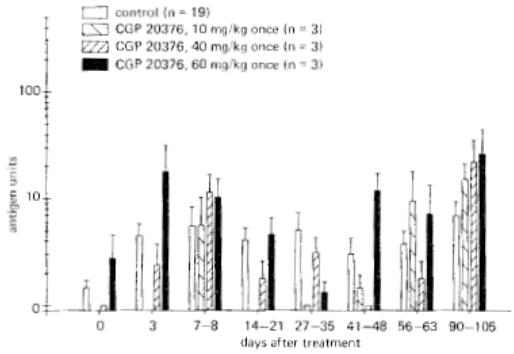

Fig. 2 Levels (mean±SE) of parasite antigen detected using the Og5A6-based ELISA in serum from groups of *Onchocerca gibsoni*-infected cattle either untreated or treated with CGP 20376

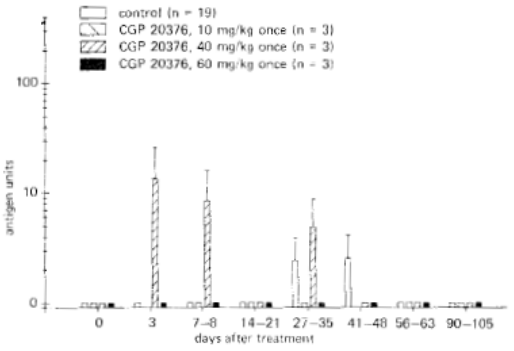

Fig. 3 Levels (mean±SE) of parasite antigen detected using the Og5C3-based ELISA in serum from groups of *Onchocerca gibsoni*-infected cattle either untreated or treated with CGP 20376

treatment groups (including controls), no statistical analysis was applied to these data.

All drugs, with the exception of CGP 6140, were microfilaricidal. The effect was dose dependent with a total dose of 60 mg/kg being 100% microfilaricidal. At a total dose of 40 mg/kg only the administration of CGP 20309 and CGP 20376 resulted in the disappearance of skin microfilariae; and only CGP 20309 approached 100% microfilaricidal effect at a total dose of 20 mg/kg. In general, the numbers of skin microfilariae began to increase again 5–8 weeks after treatment. In nodules collected approximately 3 months after treatment, there was a dramatic reduction in the total number of small morulae, advanced embryos and pretzels [terms used according to the criteria of Vankan and Copeman (1988)] in animals treated with all compounds (except CGP6 140), at doses of 40 mg/kg and above. The histopathological assessment of the nodules recovered from the trials has not yet been completed. From previous studies, however, all of these compounds (including CGP 6140 when given orally) are known to have macrofilaricidal activity against *O. gibsoni*, particularly at doses of 40 mg/kg or greater.

Discussion

The three monoclonal antibody-based assay used in this experiment were unable to predict parasite damage following the administration of proven filaricidal drugs. No consistent changes in serum antigen levels were found that could be correlated with a dose-dependent drug response. Moreover, the erratic detection of circulating antigen in sera from most treatment groups was also detected from non-treated controls and from animals treated with CGP 6140 which was not microfilaricidal or macrofilaricidal in this trial because of its subcutaneous route of administration.

A major problem with the interpretation of these data concerns the large variation in detectable parasite antigen measured in animals within identical treatment or control groups. This variability may be the result of a number of factors. Perhaps, the release of excretory/secretory antigens from parasites, such as those identified by Og5A4 and Og5A6, would not be constant; for example, a cyclic pattern of reproduction has been postulated in *O. volvulus* and *O. gibsoni* (Schulz-Key and Karam, 1986; Vankan and Copeman, 1988). The release of gut-derived antigens may also be intermittent; there is evidence both for (Franz and Büttner, 1983) and against (Franz et al. 1987; Franz and Copeman, 1988) a patent gut of *O. volvulus* and *O. gibsoni*. Furthermore, the collection of single samples days or even weeks apart can, at best, provide only a discontinuous view of the continuing real situation and may have accentuated any irregularities that were present. Finally, stored serum samples have been shown to be less accurate diagnostically in antigen detection assays than fresh samples and may have contributed to this problem (Rylatt et al., 1984). An effort to identify the reason for this variability, using these assays to monitor the antigen output from male *O. gibsoni* maintained in vitro, has been described in an accompanying paper (More and Copeman, 1991).

Future work to produce monoclonal antibody-based antigen detection assays for bovine serum (or urine) to predict filaricidal effects against *O. gibsoni* would be promoted if potential assays were screened using a defined bank of bovine sera. An effective screen for each assay could be achieved using a single 96 well plate and duplicate wells of heat-treated and untreated samples of bovine serum collected from an infected animal on weeks 4, 3, 2 and 1 prior to treatment with a known macrofilaricide, and on weeks 0.5, 1, 2, 4, 8 and 12 after treatment. Inclusion of the 4 pretreatment samples should allow identification and elimination of assays which detect antigens that normally fluctuate widely in the serum, thus reducing the sensitivity of interpretation of comparisons of pre and post treatment values. Each potentially useful assay identified should then be further evaluated with a similar range of banked sera from another 9 cattle treated with the same dose of that filaricide. The recommendation for use of this number of cattle is somewhat arbitrary but represents the number that might have been expected to yield significant results, with a level of variability similar to that obtained in the present experiment. For the same reason, inclusion of a minimum of 4 pretreatment samples has been recommended.

Unfortunately, there is no clearly indicated single macrofilaricidal candidate which should be used to create the bank of sera. It also remains to be seen if an assay able to predict parasite death following the administration of one filaricidal drug will provide comparable information for other drugs of similar efficacy but a different mode of action. Nevertheless, since successful assays are intended to provide a means of monitoring effects of chemotherapy on *O. volvulus*, it seems logical to restrict the choice of filaricides to those currently being developed for use in man; CGP 20309, CGP 20376, CGP 6140 and CGP 21833; thus providing appropriate tools for current use.

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