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Triclabendazole: new skills to unravel an old(ish) enigma

I. Fairweather*

Parasite Proteomics and Therapeutics Research Group, School of Biology and Biochemistry, The Queen's University of Belfast, Medical Biology Centre, 97, Lisburn Road, Belfast, BT9 7BL, UK

Abstract

Triclabendazole was introduced in the early 1980s for the treatment of *Fasciola hepatica* infections in livestock. Due to its high activity against immature flukes, it has become established as the principal anti-fluke drug on the market. More recently, triclabendazole has been used to treat human cases of fascioliasis and is now the drug of choice for this infection, too. Resistance to triclabendazole was first reported in 1995 in a field population in Australia and, since that time, resistant populations have been identified in several countries in Europe. Parallel to the spread of resistance has been a sharp increase in the prevalence of fascioliasis, which has been attributed largely to climate changes. Consequently, farmers are faced with an alarming scenario, as none of the other fasciolicides on the market possess such high activity against the damaging immature stages of fluke. The main aim of this review is to assess current understanding of the mechanism of action of triclabendazole against the fluke and the mechanism by which the fluke has become resistant to it. The use of triclabendazole against animal and human infections is summarized and suggestions are given on ways to deal with resistance. Gaps in the knowledge of various aspects of its use are highlighted and this may serve to open up future research areas.

Introduction

The liver fluke, *Fasciola hepatica* is a major parasite of livestock in temperate regions throughout the World. It infects 300 million cattle and 250 million sheep worldwide, causing economic losses of US $3 billion per annum (Boray, 1994). There has been a dramatic increase and spread of fascioliasis in recent years as a result of the change in climate to milder, wetter weather, and perhaps greater stock movements as well. In certain parts of the UK, for example, infection levels are running at more than 30% in cattle and more than 20% in sheep (Wolstenholme *et al.*, 2004). Fascioliasis is also emerging as a major zoonosis and is considered to be a serious health problem in some countries. Triclabendazole (TCBZ) has been the drug of choice for treating liver fluke infections in livestock for over 20 years and more recently has been used successfully to treat human cases of fascioliasis. The very success of TCBZ has almost inevitably led to the emergence of resistance to it and this could severely compromise its future use. Triclabendazole resistance does not appear to have reached the levels seen with other anthelmintics (including other benzimidazoles) but, given the heavy dependence of the livestock industry on antiparasitic drugs to maintain productivity and animal health, it is a matter for serious concern.

A review of triclabendazole is timely, therefore, to assess understanding of different aspects of its use. The topics to be addressed will be efficacy, pharmacokinetics in the host, uptake and metabolism of TCBZ by the fluke, and mechanisms of action and resistance. Strategies to deal with resistance will be discussed and future research directions will be highlighted.

Efficacy

Triclabendazole is a benzimidazole derivative, but the presence of a chlorinated benzene ring and the absence of a carbamate moiety clearly distinguish it from all other

*Fax: +44 28 90975877
E-mail: i.fairweather@qub.ac.uk

benzimidazoles (Bennett & Köhler, 1987; Lipkowitz & McCracken, 1991). It has an unusual spectrum of activity, too, in that unlike other benzimidazoles which show only marginal activity against the liver fluke, its efficacy is largely fasciolid-specific. That is, it is active against *Fasciola hepatica*, *Fasciola gigantica* (Sanyal & Gupta, 1996; Santra *et al.*, 1999) and *Fascioloides magna* (Foreyt, 1989). Triclabendazole is also active against *Paragonimus* spp. (Calvopiña *et al.*, 1998, 2003), but lacks activity against other trematodes, cestodes and nematodes. Again, this delineates it from other benzimidazoles which typically have broad-spectrum activity against a variety of helminth parasites.

Against *F. hepatica*, TCBZ shows high activity against both adult and juvenile flukes down to 1-week-old flukes (Boray *et al.*, 1983; Smeal & Hall, 1983; Turner *et al.*, 1984). At elevated dose levels, TCBZ has good activity when given to sheep 1 day post-infection (Boray *et al.*, 1983). The high activity against immature flukes is significant because they represent the migratory, tissue-invading and most damaging stages of the infection. This explains why TCBZ has become the main drug used to treat liver fluke infections. It has been shown that in sheep treated with sub-therapeutic doses of TCBZ, the pre-patent period is extended by up to 6 weeks. Moreover, flukes recovered from the treated sheep were significantly smaller than those from untreated animals (Büscher *et al.*, 1987). This suggests that in those flukes that survived exposure to TCBZ, development was delayed or retarded. Whether the stunting is permanent or not and whether it has an impact on the numbers of eggs produced per fluke were not determined. Triclabendazole is marketed under the trade names 'Fasinex' and 'Flukare'. It is also marketed in combination with other anthelmintics: with levamisole (as 'Combinex' and 'Endex'); with oxfendazole (as 'Flukazole'); with ivermectin (as 'Fasimec'); and with abamectin (as 'Flukamec'). Triclabendazole is normally given as an oral treatment, but recently a pour-on formulation ('Genesis Ultra Pour-On', in combination with abamectin) has been introduced to the market.

Pharmacokinetics

Triclabendazole is metabolized into a number of compounds (for their structures, see fig. 1). Following administration (intra-ruminal or oral), TCBZ is rapidly removed by the liver and oxidized to the sulphoxide and sulphone metabolites. Depending on the route of administration, plasma levels peak at 18–24 h (sulphoxide, TCBZ.SO) and 36–48 h (sulphone, TCBZ.SO_2); neither TCBZ nor any other metabolites can be detected in plasma (Alvinerie & Galtier, 1986; Mohammed Ali *et al.*, 1986; Hennessy *et al.*, 1987). The metabolites bind strongly (>99%) to plasma proteins, specifically albumin, and this explains why their appearance in the plasma is relatively slow. Binding will also extend their active lifespan in the host and points to oral ingestion of the compounds by the fluke, given its haematophagous behaviour. Hydroxylation of TCBZ and its two main metabolites also occurs in the liver, but the products are secreted into the bile, mainly in conjugated form. Maximum levels of the hydoxylated compounds are reached after 8 h (OH-TCBZ), 21 h (OH-TCBZ.SO) and 36 h (OH-TCBZ.SO_2) (Hennessy *et al.*, 1987). TCBZ.SO and TCBZ.SO_2 are the two main (unconjugated) metabolites in both plasma and bile (Hennessy *et al.*, 1987). Of the administered dose, ~45% is secreted in bile and (only) 6.5% excreted in urine (Hennessy *et al.*, 1987). Therefore, the fluke is exposed to

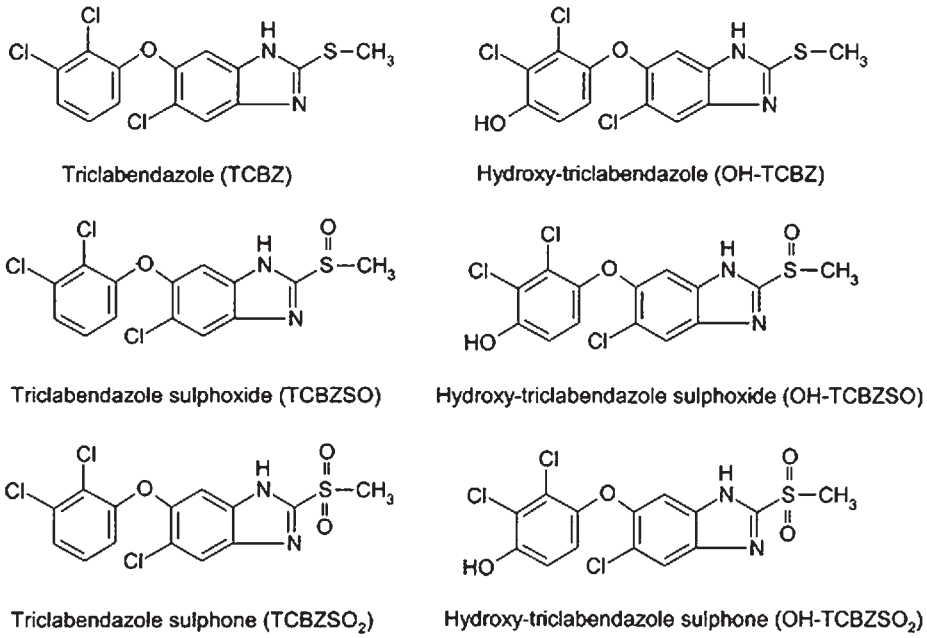

Fig. 1. Chemical structures of triclabendazole and its main metabolites.

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high levels of drugs in the bile, but whether the fluke ingests or absorbs them is not known.

Uptake and metabolism by the fluke

Two routes of drug entry into the fluke are available: oral ingestion and transtegumental diffusion. The binding of TCBZ.SO and TCBZ.SO_2 to plasma proteins suggests that they enter the fluke predominantly via the mouth. However, TCBZ and all of its metabolic products can enter the fluke by diffusion, although the diffusion of the hydroxy derivatives is lower than that for TCBZ, TCBZ.SO and TCBZ.SO_2, which showed a similar ability to enter the fluke (Mottier *et al.*, 2004). The uptake of the compounds is closely correlated with their lipophilicity (Mottier *et al.*, 2004). Diffusion of TCBZ into the fluke is less than more lipophilic compounds such as albendazole and fenbendazole; moreover, the uptake is reduced in the presence of bile (Alvarez *et al.*, 2004). Uptake of TCBZ *in vitro* occurs even when the oral route has been closed by ligation (Bennett & Köhler, 1987), which suggests that diffusion does play a role in drug uptake *in vivo*. The presence of a variety of metabolites in the bile provides the opportunity for uptake by both diffusion and oral ingestion. The fluke has been shown to be capable of metabolizing parent TCBZ to its sulphoxide and sulphone metabolites (Mottier *et al.*, 2004; Robinson *et al.*, 2004a).

Mechanism of action

There has been an assumption that TCBZ.SO is the active form of the drug, due to the parent compound being short-lived in the host and because it is the major unconjugated metabolite in both blood plasma and bile. However, the sulphone metabolite does have some activity in its own right *in vivo*: it causes a 41% reduction in worm burden when administered at 4 weeks post-infection (Büscher *et al.*, 1999). Therefore, it is possible that TCBZ action is due to the combined effect of a number of different metabolites. Having said that, most work has focused on TCBZ.SO and it is this data that will be discussed.

The precise mode of action has not yet been established. Among the suggestions put forward are uncoupling of oxidative phosphorylation (Carr *et al.*, 1993) and inhibition of protein synthesis (Stitt *et al.*, 1995). A neuromuscular action is unlikely, given that the effect is long-term at elevated drug concentrations (Fairweather *et al.*, 1984). For more complete data on TCBZ actions, see Fairweather & Boray (1999a).

Since triclabendazole is a benzimidazole compound, it might be expected that it also disrupts microtubule-based processes as a result of binding to the β-tubulin molecule, as has been shown for other benzimidazoles (Lacey, 1988). There is morphological and immunocytochemical data in support of this idea. In the tegument, for example, TCBZ.SO blocks the transport of secretory bodies from the cell body to the tegumental surface. The block occurs at the site of their formation by the Golgi complex in the cell body, in their movement through the cytoplasmic connections to the syncytium, and in their movement from the base to the apex of the syncytium (Stitt & Fairweather, 1994). This leads to the progressively severe surface damage visible externally, culminating in the total loss of the tegument (Stitt & Fairweather, 1993a). Triclabendazole sulphoxide also inhibits the mitotic division of spermatogenic cells, reducing the number of sperm produced (Stitt & Fairweather, 1992) and the stem vitelline cells, which disrupts egg formation (Stitt & Fairweather, 1996). These changes are typical of what might be expected to result from microtubule inhibition. In addition, treatment of TCBZ-susceptible flukes with TCBZ.SO leads to a loss of tubulin immunoreactivity in the tegumental syncytium (Robinson *et al.*, 2002).

It is generally accepted that benzimidazoles bind to the colchicine binding site on the β-tubulin molecule and this has been used as the basis for evaluating the relative activity of benzimidazoles. For TCBZ, the colchicine binding data is contradictory: it has been shown to cause marked inhibition of colchicine binding to purified fluke tubulin (Bennett & Köhler, 1987), but not to fluke homogenates (Fetterer, 1986), perhaps due to non-specific binding to other fluke proteins. *Fasciola hepatica* is more refractory to colchicine action than other microtubule inhibitors such as tubulozole-C (Stitt & Fairweather, 1992, 1993b) and so TCBZ may target an alternative binding site on the tubulin molecule. This may explain why more typical benzimidazoles are poorly active against *F. hepatica* and why, conversely, TCBZ lacks more broad-spectrum activity against cestode and nematode parasites. The shape of the TCBZ.SO molecule (U-, not L-, shaped) has been implicated in its limited spectrum of activity (Lipkowitz & McCracken, 1991).

Recently, a model has been put forward to identify the benzimidazole binding site on the nematode (*Haemonchus contortus*) β-tubulin molecule (Robinson *et al.*, 2004b). A typical benzimidazole compound such as albendazole sulphoxide (ALB.SO) can access the site in benzimidazole-'susceptible' worms, but not in 'resistant' worms, due to amino acid mutations in the β-tubulin molecule (Robinson *et al.*, 2004b). The β-tubulin molecule of *F. hepatica* contains a number of amino acid differences from those in *H. contortus* (Robinson *et al.*, 2001, 2002). In particular, tyrosine is present at position 200 rather than phenylalanine in TCBZ-susceptible flukes. This substitution is considered to be responsible for benzimidazole resistance in nematodes (Kwa *et al.*, 1994). The sequence changes in *F. hepatica* β-tubulin render the putative benzimidazole binding site inaccessible to ALB.SO (Robinson *et al.*, 2004b). This may help to explain why the liver fluke is more refractory to the action of typical benzimidazole drugs. Triclabendazole will not fit into the proposed benzimidazole binding site (Robinson *et al.*, 2004b) and so, if its action is directed against microtubules, it may bind elsewhere on the tubulin molecule; however, its binding site remains to be elucidated.

Triclabendazole resistance

The first identification of TCBZ resistance was made in 1995, in a field isolate of *F. hepatica* on a sheep farm in Victoria, Australia (Overend & Bowen, 1995). Triclabendazole-resistant flukes in sheep have also been reported in Co. Sligo, Ireland (Anon., 1995), in Scotland (Anon., 1998; Mitchell *et al.*, 1998) and in southwest Wales

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(Thomas *et al.*, 2000). It is the Sligo TCBZ-resistant isolate that has been used in a number of *in vivo* and *in vitro* studies, as discussed below (Coles *et al.*, 2000; Coles & Stafford, 2001; Robinson *et al.*, 2002, 2004a; Alvarez *et al.*, 2005; McCoy *et al.*, 2005). Elsewhere in Europe, TCBZ resistance has been reported in both sheep and cattle in The Netherlands (Moll *et al.*, 2000; Gaasenbeek *et al.*, 2001) and in Spain (Rojo-Vásquez, 2004). In The Netherlands, no reversion of resistance occurred after 3 years of not using TCBZ; TCBZ was replaced by closantel treatment during this time (Borgsteede *et al.*, 2005). There have been a number of anecdotal reports of resistance as well, but whether they represent genuine resistance or not has not been established. It is possible that they may be cases of product failure, perhaps due to decreased metabolism of TCBZ as a result of liver damage caused by the fluke infection, but this possibility has not been investigated.

A number of fluke isolates have been examined for their susceptibility or resistance to TCBZ. They are the Cullompton, Fairhurst (previously described as the Compton), Oberon and Sligo isolates. *In vivo*, TCBZ has a very high efficacy against the Fairhurst and Cullompton isolates (Walker *et al.*, 2004; McCoy *et al.*, 2005), but very limited efficacy against the Oberon and Sligo isolates (Coles *et al.*, 2000; Coles & Stafford, 2001; Walker *et al.*, 2004). The efficacy data has been supplemented by morphological data, using scanning and transmission electron microscopy and immunocytochemistry to evaluate the response of the isolates to treatment *in vitro* with TCBZ.SO. Tegumental disruption was more severe in the TCBZ-susceptible isolates than in the TCBZ-resistant isolates (Robinson *et al.*, 2002; Walker *et al.*, 2004). Immunocytochemical studies, using an anti-tubulin antibody, showed that tubulin immunoreactivity was abolished in the tegumental syncytium of the Cullompton isolate, but not in the Sligo isolate, even at high drug concentrations (Robinson *et al.*, 2002).

With respect to the mechanism of resistance to TCBZ, it does not appear to be associated with mutations in the β-tubulin molecule of the fluke. Tyrosine is present at position 200 in the β-tubulin from both TCBZ-susceptible and -resistant isolates (Robinson *et al.*, 2001, 2002). Moreover, no amino acid polymorphisms are present in any of the β-tubulin isotypes that have been isolated to date (L. Ryan, personal communication). This is in direct contrast to the situation in nematodes, in which the phenylalanine-tyrosine substitution at position 200 (F200Y) in the β-tubulin molecule is considered to be the most important mutation conferring resistance to benzimidazoles. Other mutations, such as the F167Y, may also contribute to resistance (Wolstenholme *et al.*, 2004).

In searching for an alternative explanation for TCBZ resistance, attention has focused on changes in the uptake and/or metabolism of TCBZ and its metabolites. Diffusion of TCBZ and TCBZ.SO into TCBZ-resistant flukes is significantly lower than in TCBZ-susceptible flukes (Alvarez *et al.*, 2005). This suggests that P-glycoprotein (Pgp)-linked drug efflux pumps may be involved in the resistance mechanism; over-expression of Pgp has been implicated in resistance to ivermectin, closantel and benzimidazoles in nematodes (Kerboeuf *et al.*, 2003; Wolstenholme *et al.*, 2004). Triclabendazole-resistant flukes have been shown to metabolize TCBZ.SO to TCBZ.SO_2 (a potentially less active metabolite) to a greater extent than TCBZ-susceptible flukes (Robinson *et al.*, 2004a). The combined effect of reduced drug uptake and more rapid drug metabolism would reduce the effective concentrations of TCBZ metabolites within TCBZ-resistant flukes. In turn, this would affect the ability of TCBZ to reach its target molecule (possibly tubulin) and so exert its fasciolicidal effect.

Strategies to deal with TCBZ resistance

Strategies to try to slow down the spread of resistance include the use of alternative fasciolicides, the use of synergistic combinations of drugs and the development of new drugs.

Use of alternative fasciolicides

Closantel, oxyclozanide, nitroxynil, clorsulon and albendazole have been shown to be active against adult TCBZ-resistant fluke (Coles *et al.*, 2000; Moll *et al.*, 2000; Coles & Stafford, 2001). These compounds are not very effective against juvenile (TCBZ-susceptible) flukes. Clinically, this is important because the migratory stages represent the most damaging phase of the disease. The result for albendazole is interesting: it could be interpreted that albendazole and triclabendazole bind at different sites on the tubulin molecule, although they share a common, microtubule-directed action.

Synergistic drug combinations

The rationale behind the concept is to try to retain the efficacy of an individual drug (in combination with another drug) when resistance to it is emerging and its efficacy is reduced when administered on its own. The use of lower-than-normal concentrations of drugs will have the benefit of reducing dose rates and reducing tissue residues of drugs. A combination of TCBZ and clorsulon at one-fifth of their recommended dose rates, and TCBZ at one-fifth and luxabendazole at one-third of their respective recommended dose rates, have been shown to be effective against 6-week-old TCBZ-resistant *F. hepatica* in sheep (Fairweather & Boray, 1999b). Despite these results, licensing regulations may severely restrict the commercial use of drug combinations.

Development of new drugs

A TCBZ derivative, designated compound alpha (α) (or compound 6) has been synthesized and tested for activity against *F. hepatica*. In the synthesis of the compound, the 3,4-dichlorophenyl group in TCBZ was replaced by a 1-naphthyl group (see fig. 2) (Hernández-Campos *et al.*, 2002). At a concentration of 15 mg kg^{-1}, compound α has been shown to be 100% effective against flukes as young as 3 days old in sheep (Hernández-Campos *et al.*, 2002). It is equally effective against both immature and mature fluke infections in cattle at a concentration of 12 mg kg^{-1}, again killing flukes as young as 3 days old (Ibarra *et al.*, 2004; Montenegro *et al.*, 2004). *In vitro*, compound α is active against newly-excysted metacercariae of *F. hepatica*

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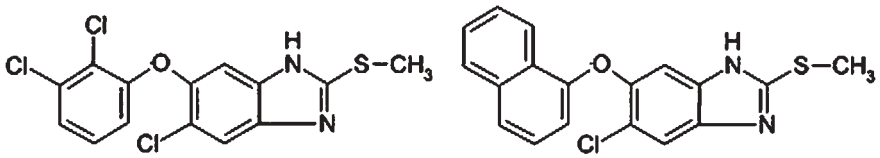

Fig. 2. Chemical structures of triclabendazole and compound 6.

at concentrations of 9.68 μM (3.3 μg ml^{-1}) and above (Hernández-Campos *et al.*, 2002). Disruption of the tegumental surface of adult and juvenile *F. hepatica* has been observed both *in vivo* and *in vitro* following treatment with compound α and its sulphoxide metabolite, respectively (Rivera *et al.*, 2004, 2005). *In vitro*, compound α. SO has been shown to cause surface changes in flukes of a TCBZ-resistant isolate of *F. hepatica* (M. McConville, personal communication), but the activity of compound α *in vivo* against TCBZ-resistant flukes has yet to be evaluated. Any commercial development of compound α would be dependent on demonstration of this activity.

Other compounds have been used recently to treat fluke infections. Nitazoxanide is a nitrothiazole derivative, originally developed for the treatment of intestinal parasitic infections. It has broad-spectrum activity against intestinal Protozoa and helminths (cestodes and nematodes), as well as some bacteria and fungi infecting animals or man (see review by Gilles & Hoffman, 2002). Human fascioliasis has been successfully treated with nitazoxanide (Rossignol *et al.*, 1998; Favennec *et al.*, 2003). However, it remains to be seen whether nitazoxanide is used more widely against *F. hepatica* as its full therapeutic value is explored more fully. The dosages required are high and the treatment period long, so its use may be restricted to cases of failure with TCBZ. A novel fasciolicide, 'Mirazid', has been registered for use against fascioliasis by the Egyptian Ministry of Health. It is derived from myrrh and has been shown to be effective against *F. hepatica* in sheep and humans (Haridy *et al.*, 2003; Abo-Madyan *et al.*, 2004). There have been claims that Mirazid is also active against schistosomes (Sheir *et al.*, 2001; Soliman *et al.*, 2004). However, other studies have failed to reproduce these results and showed that the cure rate was relatively lower than that with praziquantel (Botros *et al.*, 2004, 2005). This suggests that Mirazid is not likely to play a significant role in the treatment and control of schistosomiasis. Similar caution may need to be exercised when assessing its use against fascioliasis. Metronidazole, an imidazole derivative used against infections with bacteria, protozoans and some nematodes, has also been used successfully to treat patients who had not responded to TCBZ (Mansour-Ghanaei *et al.*, 2003).

Use of triclabendazole to treat human fascioliasis

Fascioliasis is becoming an increasingly significant zoonotic infection of man. It has one of the widest latitudinal, longitudinal and altitudinal distributions of any parasitic disease (Mas-Coma *et al.*, 1999). Cases have been described in 61 countries in five continents. High prevalences occur in Andean countries in South America (Bolivia, Chile, Ecuador, Peru), in the Caribbean (Cuba), northern Africa (Egypt), the near East (Iran), South-East Asia and western Europe (Portugal, Spain, France) (Mas-Coma *et al.*, 1999). In hyperendemic areas it is becoming a major threat to public health. Estimates of people infected range from 2.4 million (Crompton, 1999) to 17 million (Hopkins, 1992), while the number of people at risk of infection has been estimated at 180 million (Farag, 1998). Following its successful use in the treatment of fascioliasis in livestock, a number of studies showed that triclabendazole was effective against *F. hepatica* in humans (e.g. Wessely *et al.*, 1988; Laird & Boray, 1992; Apt *et al.*, 1995). This led to it being placed on the WHO Model List of Essential Medicines in 1998 (Savioli *et al.*, 1999). It has now become the drug of choice for human fascioliasis and the human formulation is marketed as 'Egaten'. Triclabendazole is administered as a single dose, though more severe cases may require more than one dose (Richter *et al.*, 2002; Talaie *et al.*, 2004). As mentioned earlier, triclabendazole is also used for the treatment of human paragonimiasis (Calvopiña *et al.*, 1998, 2003).

Conclusions/future directions

It is clear that many aspects of TCBZ use remain to be elucidated. For example, the mechanisms of action and resistance and the pharmacodynamic interactions between the fluke and the various metabolites of TCBZ. The true extent of TCBZ resistance in the field is also unknown. Tests for resistance are needed urgently in order that surveys can be carried out in the field to properly assess the problem. For the immediate future the tests will be simple parasitological ones, probably based on the egg stage. As more is learnt about the targets of TCBZ action in the fluke and the molecules involved, this may reveal markers that could be used in more sensitive molecular tests in the future. Given the dominant position of TCBZ in the market-place, the development and spread of resistance to it is a matter of considerable concern. The problem needs to be addressed before the situation becomes as serious as it is with other, more broad-spectrum anthelmintics. Also, before resistance starts to appear in human isolates of fluke.

Acknowledgements

This review is affectionately dedicated to Dr Joe Boray as, in many respects, triclabendazole is Joe's 'baby'. He

was involved in the original development and testing of TCBZ and in monitoring TCBZ-resistant fluke populations; also, in the testing of combinations of drugs to deal with resistance. He tells me that, back in 1972, he selected the first structure that led to TCBZ from a group of insecticides used as moth-proofing agents. Who knows, just as moths are attracted to the light, perhaps TCBZ (under the spotlight of the scientific community) will start to reveal its secrets and succumb to the flame. . .. Anyway, Joe, here's the story so far.

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