**Echinococcus** metacestodes as laboratory models for the screening of drugs against cestodes and trematodes

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(Received 12 June 2009; revised 23 July 2009; accepted 25 July 2009; first published online 21 September 2009)

**SUMMARY**

Among the cestodes, *Echinococcus granulosus*, *Echinococcus multilocularis* and *Taenia solium* represent the most dangerous parasites. Their larval stages cause the diseases cystic echinococcosis (CE), alveolar echinococcosis (AE) and cysticercosis, respectively, which exhibit considerable medical and veterinary health concerns with a profound economic impact. Others caused by other cestodes, such as species of the genera *Mesocestoides* and *Hymenolepis*, are relatively rare in humans. In this review, we will focus on *E. granulosus* and *E. multilocularis* metacestode laboratory models and will review the use of these models in the search for novel drugs that could be employed for chemotherapeutic treatment of echinococcosis. Clearly, improved therapeutic drugs are needed for the treatment of AE and CE, and this can only be achieved through the development of medium-to-high throughput screening approaches. The most recent achievements in the in *vitro* culture and genetic manipulation of *E. multilocularis* cells and metacestodes, and the accessibility of the *E. multilocularis* genome and EST sequence information, have rendered the *E. multilocularis* model uniquely suited for studies on drug-efficacy and drug target identification. This could lead to the development of novel compounds for the use in chemotherapy against echinococcosis, and possibly against diseases caused by other cestodes, and potentially also trematodes.

Key words: Cystic echinococcosis (CE), alveolar echinococcosis (AE), *Echinococcus granulosus*, *Echinococcus multilocularis*, in *vitro* culture, chemotherapy.

**INTRODUCTION**

The genus *Echinococcus* includes seven to nine described species (Nakao et al. 2007; Varcasia et al. 2008). Of these, *Echinococcus multilocularis* (the small fox tapeworm) is the most pathogenic, and *E. granulosus* (the small dog tapeworm) represents the most common species (Thompson, 1986). Both cause life-threatening diseases of serious public health and economic concern worldwide (McManus et al. 2003). Alveolar echinococcosis (AE), caused by *E. multilocularis*, is largely restricted to the Northern hemisphere, such as Central Asia, Russia, Western China, Europe and Japan. Infections with *E. granulosus*, the causative agent of cystic echinococcosis (CE), occur globally, and mostly in the Mediterranean area, Central Europe, South America, Africa, Central Asia, and CE exists as an imported disease in Western Europe and the USA (Schantz et al. 1995; Eckert and Deplazes, 2004). 3.6 million disability-adjusted life years (DALYs) are lost due to CE (Craig et al. 2007), showing that the impact of the disease is comparable to onchocercosis and African trypanosomosis (Budke et al. 2006). AE and CE are diseases of communities that often lack essential resources, thus the development of new drugs against these diseases has not been a major focus of the pharmaceutical industry. First, because the population affected and the number of cases as such do not represent a promising market. Secondly, there has been an inherent lack of in *vitro* culture systems that would allow cost-effective high-throughput drug screening. However, the recent achievements in the establishment of in *vitro* cultivation systems, especially for *E. multilocularis* metacestodes, has now opened the door for medium-to-high-throughput drug screening possibilities. These developments could have considerable impact not only for the treatment of echinococcosis, but also for other diseases caused by cestodes such as *Taenia*, *Hymenolepis*, *Mesocestoides* and *Spirometra* and, potentially, trematodes.

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**ECHINOCOCCUS: BIOLOGY AND DISEASE**

*E. multilocularis* and *E. granulosus* share some distinct features in their life cycle. The adult worms live...
in the intestine of their respective final host (dogs for *E. granulosus*, foxes, dogs and cats for *E. multilocularis*) where sexual reproduction and subsequent egg production takes place. Faecal shedding spreads the eggs into the environment, where they are accidentally taken up by suitable intermediate hosts, such as small rodents for *E. multilocularis*, and cattle and sheep for *E. granulosus*. These eggs contain the first larval stage, the oncosphere, and during stomach passage, the oncosphere is activated and leaves the protective egg. It actively penetrates the intestinal lining, and migrates via blood and lymphatic vessels to the visceral organs. These are primarily the liver for *E. multilocularis*, and the liver, lung and other target organs in the case of *E. granulosus*. There, these oncospheres develop into metacestodes, which represent the second larval stage. Within these metacestodes, protoscolex development takes place, and upon oral uptake by the respective final host, protoscoleces attach to the intestinal epithelium and develop into adult worms, thus concluding the life cycle (Rausch, 1995).

Metacestodes of both species are fluid-filled vesicles, and represent the disease-causing stage. They are structured into an inner cellular and an outer acellular compartment (Fig. 1). The outer, acellular surface of the metacestode is formed by the laminated layer, a carbohydrate-rich structure.
The laminated layer covers the entire metacestode surface, and is much more prominent in *E. granulosus* metacestodes (Morseth, 1967; Gottstein and Hemphill, 1997). The actual larval tissue is formed by the germinal layer, which itself is composed of a population of different cell types (Figs. 1 and 2). One part consists of the tegument, which is directly associated with the inner surface of the laminated layer. It is characterized by microvilli-like extensions, microtriches, which protrude into the matrix of the laminated layer and thereby increase the resorbing surface of the parasite (Fig. 2). In addition, the germinal layer contains highly differentiated cell types including connective tissue, muscle cells, and glycogen storage cells, as well as many undifferentiated cells (Fig. 2).

The fully mature *E. granulosus* metacestode (i.e. hydatid cyst) is a single-chambered or septate unilocular cyst that shows expansive growth and thereby causes compression of neighbouring tissue, tissue damage and organ dysfunction (Kern, 2006). Accidental or traumatic cyst rupture can result in release of cyst fluid and dissemination of protoscoleces, leading to anaphylactic reactions and metastases (secondary echinococcosis) (Stey and Jost, 1993). *E. granulosus* metacestodes are surrounded by a host fibrous capsule, the adventitial layer, which is composed of host connective tissue.

In *E. multilocularis* infection, metacestode development exhibits different characteristics. There is no limiting host-tissue barrier comparable to the adventitial layer. The metacestode tissue represents a multivesicular structure that is reproducing...
asexually, by formation and budding of daughter vesicles, with progressive tumour-like growth (Ali-Khan et al. 1983). This leads to the formation of a large and heterogeneous parasitic mass that is intermingled with host connective tissue, proliferative in the periphery, and often necrotic in the centre (Gottstein and Hemphill, 1997). Metastases formation may occur in other organs due to the release of germinal layer cells into the blood or lymph system (Ali-Khan et al. 1983; Eckert et al. 1983; Mehlhorn et al. 1983). Thus, AE can resemble a benign malignant tumour (Vuitton, 2009). Protoscolex development in humans has been rarely observed.

**BENZIMIDAZOLES FOR THE CHEMOTHERAPEUTIC TREATMENT OF CYSTIC ECHINOCCOCCOSIS (CE) AND ALVEOLAR ECHINOCCOCCOSIS (AE)**

For many years, the preferred treatment strategy for CE has been radical resection of the parasite mass (Kern, 2003, 2006). Other options include image-guide percutaneous treatment (PAIR=puncture, aspiration, injection, reaspiration) (Brunetti et al. 2004) and chemotherapy (El-On, 2002). Both surgery and PAIR are always accompanied by chemotherapy and, for inoperable cases, chemotherapy remains the only option. These cases include patients exhibiting cysts in crucial organs such as spine, pelvis and other sites that are not easily accessible, or patients with multiple cysts in several organs. The currently used drugs are mebendazole and albendazole. These drugs clearly have the potential to kill metacestodes, and to cure the patients. Benimidazoles exert their action by binding to tubulin, inducing microtubule depolymerization and inhibiting polymerization of this essential cytoskeletal element (reviewed by Hemphill and Müller, 2009). However, the efficacy of these benzimidazoles depends on size and type of cyst (benzimidazoles are more effective against smaller cysts), on the age of the patient (these drugs work better in younger than in older patients), and on which organ is affected (e.g. cysts localized in bones are less susceptible to chemotherapy than those in liver and lungs). The duration of treatment plays a crucial role, with prolonged drug administration producing the most favourable results for the patients (Horton, 1997; Franchi et al. 1999; Vutova et al. 1999). Praziquantel, a heterocyclic pyrazinoisoquinoline derivative, has been proposed to be used alongside benzimidazoles in CE-patients. Praziquantel is well tolerated, less toxic and better absorbed than albendazole. Praziquantel is used against the adult stages of *Echinococcus* and many other cestodes, and was shown to exhibit a high efficiency against protoscoleces (Morris et al. 1986) and metacestodes in animal experiments (Urrea-Paris et al. 1999, 2001). The mode of action of praziquantel is a matter of debate. Respective studies were mostly carried out with schistosomes, where praziquantel represents the only drug that is currently marketed. Possibilities of praziquantel toxicity include its actions on nucleoside uptake (Angelucci et al. 2007), inhibition of phospho-inositide turnover (Wiest et al. 1992), binding to parasite actin (Tallima and El Ridi, 2007) and the parasite myosin light chain, and possibly inhibiting its functional activity (Gnanasekar et al. 2009), interference in glutathione-S-transferase activity (McTigue et al. 1995), and stimulation of Ca\(^{2+}\) entry through voltage-operated Ca\(^{2+}\) channels (VOCCs) (Kohn et al. 2003; Jeziorski and Greenberg, 2006). The most compelling evidence for the involvement of VOCCs was recently provided by Nogi et al. (2009): they used the free-living flatworm *Dugesia japonicum* and investigated regeneration of fragments excised from this planarian that have the ability to reform a complete body plan, an ability that is driven by a totipotent population of stem cells called neoblasts. In the presence of praziquantel, however, this regeneration process yielded complete duplication of the entire anterior-posterior axis, resulting in two-headed organisms with duplicated nervous and organ systems. This effect of praziquantel was selectively ablated by *in vivo* RNAi of VOCC beta-subunits, but not by knock-down of alpha-subunits. At higher doses of praziquantel, knock-down of VOCC beta-subunit also conferred resistance to praziquantel, confirming the critical involvement of this beta subunit in the action of the drug.

A combined treatment regimen with albendazole and praziquantel given during the month prior to surgery of *E. granulosus*-infected patients increased the number of human patients with non-viable protoscoleces as compared to therapy with albendazole alone (Cobo et al. 1998). Thus, praziquantel is regarded as useful in cases where cyst content is spilled during surgery.

For the treatment of AE, surgery and chemotherapy are the two only treatment options (Ammann and Eckert, 1995). Spontaneous cure of AE, leading to calcified lesions, is possible, but it is not known how commonly this occurs (Gottstein and Hemphill, 1997; Vuitton, 2009). Radical surgery of viable lesions is carried out if possible, but can be difficult to achieve, and only about one third of all cases are actually operated on. In contrast to *E. granulosus* metacestodes, *E. multilocularis* metacestodes are almost exclusively located in the liver. However, metastases can occur, also involving distant sites including lungs, spleen and brain. Chemotherapy should last for at least 2 years post-surgery, and monitoring of patients should be continued for 10 years. Inoperable cases must undergo long-term chemotherapy, often life-long (Reuter et al. 2000; 2004; Vuitton, 2009). The experiences with long-term use of benzimidazoles has had an enormous
impact on the use of surgery. Generally, palliative surgery should be avoided and replaced by benzimidazole treatment alone or combined with percutaneous or perendoscopic interventions to treat biliary or vascular complications (Bresson-Hadni et al. 2000, 2006; Vuitton, 2009). Extensive animal experimentation and clinical experience in human patients have both demonstrated that albendazole and mebendazole exhibit a parasitostatic rather than parasiticidal effect against *E. multilocularis* metacestodes (Reuter et al. 2004; Vuitton, 2009). Thus benzimidazoles only prevent parasite growth, and the recurrence rates after interruption of therapy are high. Nevertheless, clinical studies have shown that chemotherapy has significantly increased the 10-year survival rate of inoperable or non-radically operated AE patients from 6–25% to 80–83% (Hemphill et al. 2007).

Benzimidazoles are generally well tolerated, but problems can occur. Adverse reactions include hepatotoxicity, alopecia, gastrointestinal disturbances and leukopenia, which is sometimes severe and irreversible (Horton, 1997). Risks also include embryotoxicity and teratogenicity. In order to improve bioavailability, liposome-entrapped formulations and emulsions of albendazole have been tested (Wen et al. 1996; Chai et al. 2004). The use of cimetidine is discouraged, since it increases intestinal absorption (Wen et al. 1996; Schipper et al. 2000), which in turn could result in toxic effects.

Major problems associated with benzimidazoles are the intra- and inter-individual variations of the pharmacokinetics, which make it necessary to measure albendazole sulphoxide and mebendazole plasma levels on a regular basis in order to adjust drug dosage and to avoid toxicity (Vuitton, 2009). This is only possible in specialized facilities, and thus precludes the use of benzimidazoles in those endemic areas where efficient drugs would be mostly needed. In addition, it is unknown whether there is a stage-specific response to exposure to benzimidazoles, and the actual concentration at the site where the drug exerts its action is not known. These pharmacological uncertainties, together with the difficulties in assessing metacestode viability in humans, show that novel and improved chemotherapeutical tools are needed in order to optimize the treatment of CE and AE.

**THE ROLE OF IN VITRO CULTURE IN DRUG DISCOVERY**

In order to identify novel potential alternatives for chemotherapy against echinococcosis, the strategy used most commonly has been whole organism screening of *E. multilocularis* and *E. granulosus* metacestodes and/or protoscolecies. In most cases, researchers have focused on broad-spectrum anti-infective agents and anti-cancer compounds, many of which also exhibited reasonable efficacy against *Echinococcus* (Hemphill and Müller, 2009).

*In vitro* chemotherapy studies on CE have mostly, but not exclusively, been carried out on protoscolecies, since these are easily obtained from cysts of an infected animal. The metabolism, and thus drug-susceptibility, of *E. granulosus* protoscolecies might however be different from the metacestode stage tissue. As an example, praziquantel is highly active against adult tapeworms and against protoscolecies, but only of limited efficacy against metacestodes. Conversely, albendazole is less active against protoscolecies, but of significantly higher efficacy against metacestodes (Taylor et al. 1989).

Infection of laboratory animals with *E. granulosus* protoscolecies results in the development of metacestodes, which mimics the process of secondary hydatid disease in humans, and this differentiation process can also be achieved *in vitro* (Walker et al. 2004). *E. granulosus* metacestodes do not proliferate *in vivo* but, *as in vivo*, show a marked increase in size. Thus, extensive animal experimentation is necessary for constant supply of metacestode material. In contrast, *E. multilocularis* metacestodes, besides increasing in size, also proliferate asexually and, provided with the corresponding nutrients and growth factors, will form new vesicles either endogenously (from within the germinal layer) or exogenously (by budding of daughter metacestodes from older vesicles; Fig. 3). Thus, the fact that *E. multilocularis* metacestodes proliferate *in vitro* has rendered this model suitable for the experimental assessment of the effects of chemotherapeutically interesting compounds.

Historically, the *in vitro* culture of *E. multilocularis* metacestodes was achieved by dissection of infected rodents and placing small pieces of infected tissue or vesicle suspension into a suitable culture medium at 37 °C. After a few weeks, newly formed metacestodes emerged, which were infective when re-introduced back into rodents (Hemphill and Gottstein, 1995). These were co-culture systems, as parasites could be maintained for several months in the presence of feeder cells, and in the absence of feeder cells, metacestodes degenerated within a few weeks. Jura et al. (1996) introduced a second *in vitro* co-culture system, which was based on co-incubation of homogenized parasite tissue obtained from infected rats with primary rat hepatocytes embedded in a collagen layer. Although useful in many instances (reviewed in Hemphill et al. 2002, 2007; Brehm and Spiliotis, 2008a), these co-culture systems provided only moderate yields and only a very limited number of drugs could be studied. Thus, the amount of metacestodes that could be generated was not sufficient for larger scale investigations.

Recently, Spiliotis and Brehm (2008) have overcome this problem and established a large-scale cultivation system which utilizes homogenized
metacestode tissue that is incubated in liquid culture together with rat hepatoma feeder cells. Using this system, high numbers of metacestodes with comparable sizes can be generated within a few weeks of incubation. Thus, a major task of feeder cells appears to be the provision and secretion of growth factors that are needed by the parasites to undergo proliferation, growth and differentiation. *E. multilocularis* metacestodes possess several receptors that interact with human epidermal growth factor (EGF), insulin, transforming growth factor-beta and steroid hormones (Brehm et al. 2006; Brehm and Spiliotis, 2008b), and these most likely allow the parasite to communicate with its host and adapt to alterations in living conditions, regulating gene expression to its favour. Of course, these receptors, and associated signalling pathways within the parasite, represent interesting targets for potential chemotherapeutical intervention (Brehm et al. 2006; Gelmedin et al. 2008). In addition, Spiliotis et al. (2004) developed the methodology to culture *E. multilocularis* metacestodes axenically in the absence of feeder cells, under reducing and anaerobic conditions. In the presence of oxygen, these metacestodes degenerate within few weeks (Spiliotis et al. 2004), illustrating the high sensitivity of *E. multilocularis* metacestodes to oxygen intermediates. The specific adaptation of the mitochondrial respiratory system to anaerobic environments has been shown also for *E. multilocularis* protoscoleces (Matsumoto et al. 2008). Thus, another important task of feeder cells is to remove toxic oxygen intermediates (Brehm and Spiliotis, 2008a).

**METHODS FOR THE ASSESSMENT OF ANTI-ECHINOCOCCAL DRUG CANDIDATES**

By using animal experimentation, the primary assessment of anti-echinococcal drug candidates has often been performed in mice or gerbils, through evaluation of parasite mass and/or health parameters of the host. This has led to the extensive use of animal experimentation, but has often yielded inconclusive results (reviewed in Siles-Lucas and Hemphill, 2002). More recently, *in vitro* cultured *Echinococcus* metacestodes have been increasingly used for the primary assessment of drug susceptibilities (Hemphill et al. 2002, 2007).

A major problem associated with drug-efficacy assessment has always been the monitoring of the actual metacestode viability following *in vitro* drug treatment. In a primary evaluation, the effects of *in vitro* drug treatment can be assessed mainly by visual inspection of morphological alterations and light-microscopy. SEM and TEM have also been used extensively to investigate tissue damage in more detail (Ingold et al. 1999; Stettler et al. 2003; Naguleswaran et al. 2006; Spicher et al. 2008a; see also Fig. 2E, F), and nuclear magnetic resonance spectroscopy (NMR) has been applied to investigate metabolic changes imposed upon the parasites by drug treatments (Ingold et al. 1999). Others have
attempted to assess parasite viability and growth by the quantification of the expression of molecular marker genes such as 14-3-3 and 11/3-10 (Matsumoto et al. 2006). Visual inspection relies on subjective observations and thus requires experienced personnel, while other techniques such as SEM, TEM, NMR and RT-real time PCR are intrinsically time-consuming and expensive. Although these approaches have lead to the identification of several compounds with reasonable activities in the micromolar range in vitro (see below), it has not been possible to implement these techniques in a cost-effective manner at a larger scale, and they have not always produced conclusive results. For instance, in vitro culture of E. multilocularis metacestodes in the presence of albendazole sulphoxide for 14 days resulted in altered composition of vesicle fluid metabolites as assessed by NMR, and complete destruction of the metacestode structural integrity as visualized by SEM and TEM (Ingold et al. 1999). However, bioassay (re-inoculation of treated parasite material into mice) subsequently showed that the parasite had not been killed completely (Stettler et al. 2003). This was confirmed by Reuter et al. (2006), and illustrates the difficulties in viability assessment following drug treatment.

Although several potentially interesting compounds were identified, and combinations of some of these drugs with albendazole led to slightly improved treatment efficacy in experimentally infected mice or gerbils, none of these compounds exhibited improved activities compared to albendazole (reviewed in Hemphill et al. 2007). Thus, many more drugs and/or compound classes should be investigated, and there is an urgent need for a reliable, but also easy-to-handle and rapid in vitro drug screening assay for the identification of chemotherapeutically interesting compounds in vitro. Attempts to develop screening assays based on the use of vital dyes employed for the assessment of cell viability, such as Alamar blue and Trypan blue, were undertaken. They worked well for protoscoleces viability assessment (Walker et al. 2004), but they were not practical when used with metacestodes, as these dyes tend to bind to components of the laminated layer (M. Spicher et al. unpublished observations). The MTT viability assay, which is based on the activity of mitochondrial reductase that reduces yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyldiazotrazoliumbromide to purple formazan in living cells, worked well for E. multilocularis metacestodes in an experimental setting (Emery et al. 1995), but was not found to be useful for the assessment of larger numbers of compounds (A. Hemphill, unpublished observations).

Another biomarker-based approach to develop such a screening assay could be to identify specific enzyme activities in culture supernatant obtained from drug-treated parasites, which release vesicle fluid components once they are damaged or structurally impaired upon exposure to an active compound. Such biomarkers indicating damage are not present in culture supernatants of untreated metacestodes. E. multilocularis metacestodes are basically fluid-filled vesicles that are surrounded by an outer, acellular laminated layer, and the inner surface of this laminated layer is delineated by the actual parasite tissue, the germinal layer. The germinal layer secretes the components of the laminated layer towards the metacestode periphery into the laminated layer, and also secretes and/or releases metabolites into the vesicle fluid. One enzyme that represents an intrinsic component of the vesicle fluid, and which is also found on the laminated layer, is alkaline phosphatase (AP; Sarciron et al. 1991; Lawton et al. 1997). The detection of AP activity in medium supernatants of drug-treated metacestode cultures has been proposed as a method to screen for active drugs (Stettler et al. 2001). AP-activity was indeed increased in culture supernatants of metacestodes treated with nitazoxanide (Stettler et al. 2003), 2-methoxyestradiol and artemisinin-derivatives (Spicher et al. 2008a,b), but the sensitivity of this assay was not always satisfactory (Gelmedin et al. 2008; A. Hemphill, unpublished observations). Thus, detection of AP activity does not represent a reliable measure for drug screening in vitro. Another enzyme, phosphoglucose isomerase, released by dying E. multilocularis metacestodes, was found to be a promising marker that allows assessment of parasite viability of drug-treated E. multilocularis metacestodes. The potential of this screen for high-throughput assays will be published soon (B. Stadelmann et al. unpublished observations).

Parasite-derived biomarkers could also play a major role for monitoring drug efficacy in patients suffering from echinococcosis during drug trials. For instance, it was previously shown that the serological response of patients against E. multilocularis alkaline phosphatase could reflect viability following surgery and/or chemotherapy (Sarciron et al. 1997). Such immunological tests are dependent on the availability and specificity of antigens and do not always provide clear-cut results; the same applies to molecular tests, such as PCR, which require specific primers. Thus, more recently, disease-specific biomarkers have been identified by metabolic profiling employing 1H nuclear magnetic resonance spectroscopy (NMR). This technology delivers an overview of the metabolic composition of biofluids and tissues in diseased versus non-diseased individuals, and such studies have provided the means for detection and differentiation of e.g. coronary heart disease and schizophrenia (Ordovas and Moser, 2006; Holmes et al. 2006). Metabolic profiling has been carried out in rodents infected with S. mansoni (Wang et al. 2004), S. japonicum (Wang et al. 2006), T. spiralis (Martin et al. 2006), Trypanosoma brucei brucei (Wang et al.
A selection of drugs and potential drug candidates against cystic hydatid disease caused by Echinococcus granulosus is provided in Table 1. In many instances, in vitro studies on compounds active against E. granulosus have employed protoscoleces. Experimental prophylactic therapy of E. granulosus protoscoleces was carried out as a model that would mimic spillage during surgery, by treating protoscoleces with praziquantel (Urrea-Paris et al. 2001) or a combination of praziquantel and albendazole (Casado et al. 2001) prior to injection into mice. The combination of albendazole and praziquantel has been used successfully in the treatment of human CE (Cobo et al. 1998). Other promising compounds with in vitro protoscolicidal actions against E. granulosus were cetrimide (Frayha et al. 1981) and the ionophore monensin (Rogan and Richards, 1986), but these drugs were rather ineffective against metacercoides. Levamisole and ivermectin, which are classically used against nematode infections, exhibited in vitro activities similar to benzimidazoles (Martinez et al. 1999; Elissondo et al. 2009). The direct injection of ivermectin into E. granulosus cysts in laparotomised patients has also shown parasiticidal effects (Ochieng’-Mitul and Burt, 1996). The combination of ivermectin plus albendazole has shown synergistic effects in infected mice (Moreno et al. 2002). Benzimidazoles vary considerably with regard to their protoscolicidal action. Albendazole and albendazole sulphoxide have been shown to be active against E. granulosus protoscoleces in vitro (Chinery and Morris, 1986; Morris et al. 1987; Perez-Serrano et al. 1994), but the in vitro protoscolicidal action of these drugs is rather slow and requires a longer incubation period compared to mebendazole (Morris et al. 1987; Rodriguez-Caabeiro et al. 1989) or oxendazole, the major fenbendazole sulphoxide metabolite. Against E. granulosus infection in rodents, a combination of fenbendazole and netobimin (Garcia-Llamazare et al. 1997) showed synergistic effects, allowing the treatment of cystic echinococcosis (CE).

### Table 1. A selection of drugs and potential drug candidates against cystic hydatid disease caused by Echinococcus granulosus.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Activity in humans</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxfendazole</td>
<td>Not assessed</td>
<td>Blanton 1988; Dueger et al. 1999; Gavidia et al. 2009</td>
</tr>
<tr>
<td>Flubendazole</td>
<td>Yes – cysts, protoscoleces</td>
<td>Ellissondo 2006; 2007</td>
</tr>
<tr>
<td>Albendazole + praziquantel</td>
<td>Yes – rodents</td>
<td>Cobo et al. 1998; Casado et al. 2001</td>
</tr>
<tr>
<td>Albendazole + ivermectin</td>
<td>Yes – rodents</td>
<td>Moreno et al. 1999; Martinez et al. 1999; Ochieng’-Mitul and Burt, 1996; Elissondo et al. 2009</td>
</tr>
<tr>
<td>Monensin</td>
<td>Not assessed</td>
<td>Garcia-Llamazare et al. 1997</td>
</tr>
<tr>
<td>Levamisole</td>
<td>Not assessed</td>
<td>Martı́nez et al. 1999</td>
</tr>
<tr>
<td>Ivermectin</td>
<td>No – rodents</td>
<td>Ochieng’-Mitul and Burt, 1996; Martinez et al. 1999; Elissondo et al. 2009</td>
</tr>
<tr>
<td>Nitazoxanide</td>
<td>No – sheep</td>
<td>Palomino and Burt, 1996; Morena et al. 2009</td>
</tr>
<tr>
<td>Cetrimide</td>
<td>Yes – protoscoleces</td>
<td>Frayha et al. 1981; Colebrook 2004</td>
</tr>
<tr>
<td>Monensin</td>
<td>No – rodents</td>
<td>Garcia-Llamazare et al. 1997</td>
</tr>
<tr>
<td>Ivermectin + albendazole</td>
<td>No – rodents</td>
<td>Garcia-Llamazare et al. 1997</td>
</tr>
</tbody>
</table>

### Cystic echinococcosis (CE)

A selection of drugs and potential drug candidates against CE is provided in Table 1. In many instances, in vitro studies on compounds active against E. granulosus have employed protoscoleces. Experimental prophylactic therapy of E. granulosus protoscoleces was carried out as a model that would mimic spillage during surgery, by treating protoscoleces with praziquantel (Urrea-Paris et al. 2001) or a combination of praziquantel and albendazole (Casado et al. 2001) prior to injection into mice. The combination of albendazole and praziquantel has been used successfully in the treatment of human CE (Cobo et al. 1998). Other promising compounds with in vitro protoscolicidal actions against E. granulosus were cetrimide (Frayha et al. 1981) and the ionophore monensin (Rogan and Richards, 1986), but these drugs were rather ineffective against metacercoides. Levamisole and ivermectin, which are classically used against nematode infections, exhibited in vitro activities similar to benzimidazoles (Martinez et al. 1999; Elissondo et al. 2009). The direct injection of ivermectin into E. granulosus cysts in laparotomised patients has also shown parasiticidal effects (Ochieng’-Mitul and Burt, 1996). The combination of ivermectin plus albendazole has shown synergistic effects in infected mice (Moreno et al. 2002). Benzimidazoles vary considerably with regard to their protoscolicidal action. Albendazole and albendazole sulphoxide have been shown to be active against E. granulosus protoscoleces in vitro (Chinery and Morris, 1986; Morris et al. 1987; Perez-Serrano et al. 1994), but the in vitro protoscolicidal action of these drugs is rather slow and requires a longer incubation period compared to mebendazole (Morris et al. 1987; Rodriguez-Caabeiro et al. 1989) or oxendazole, the major fenbendazole sulphoxide metabolite. Against E. granulosus infection in rodents, a combination of fenbendazole and netobimin (Garcia-Llamazare et al. 1997) showed synergistic effects, allowing the
administration of lower drug dosages. Oxfendazole, like albendazole, is a benzimidazole, used in veterinary medicine for the treatment of nematode infections, and has a similar antimicrobial spectrum but a longer half-life. Experimental treatments of naturally *E. granulosus*-infected sheep and goats suggested that oxfendazole may be as efficacious as albendazole, but does not require daily uptake of the drug because of its prolonged bioavailability (Blanton et al. 1988; Duenger et al. 1999). Nitazoxanide, a nitro-thiazole-analogue (Hemphill et al. 2006) induced severe damage to *E. granulosus* protoscoleces and the germinal layer of *E. granulosus* metacestodes within few days of *in vitro* culture (Walker et al. 2004). Nitazoxanide treatment was not effective against experimental *E. granulosus* infection in sheep, but oxfendazole treatment, and a combination of oxfendazole and nitazoxanide, significantly decreased the number of fertile cysts and increased the number of degenerated cysts (Gavidia et al. 2009). On the other hand, a recent case report has suggested beneficial effects of nitazoxanide in the treatment of refractory bony hydatid disease in a human patient (Winning et al. 2009). This patient had been suffering from progressive disease despite treatment with albendazole and praziquantel, and the clinical response on nitazoxanide treatment showed marked improvement in the soft tissue cysts, with stable disease in the bony pelvis. Although further studies are required, this report suggests that nitazoxanide may be an effective treatment option in CE, particularly in patients with progressive disease who are receiving conventional treatment (Winning et al. 2009).

Profound protoscolicidal activity was also reported for another benzimidazole-derivative, flubendazole (Elissondo et al. 2006, 2009). Further studies subsequently showed that flubendazole also exhibited anti-*E. granulosus* metacestocidal activities *in vitro* and *in vivo* in experimentally infected mice (Elissondo et al. 2007). The same authors, together with Kammerer and Perez-Esandi (1975), showed that thymol, one of the major components of the essential oils of *Thymus*, was found to exhibit substantial protoscolicidal activity (Elissondo et al. 2008).

Cyclosporin A, employed mainly as an immunosuppressant during the management of organ transplants, also exhibits anti-echinococcal activity. The administration of cyclosporin A in five consecutive daily doses, beginning 2 days prior to the infection of mice with *E. granulosus* protoscoleces, resulted in a significant reduction in cyst numbers and cyst masses measured at 20 weeks after infection. However, no changes in cyst mass and numbers were recorded when the drug was administered 18 weeks after infection, but the wet weight was decreased by 42% compared with untreated controls. Ultrastructural examination of the germinal membrane and laminated layer of late-treated *E. granulosus* revealed abnormalities in all cysts studied whereas control and early-treated hydatids were normal (Hurd et al. 1993). Cyclosporin A has also been shown to affect *E. granulosus* protoscoleces *in vitro* (Colebrook et al. 2004).

**Alveolar echinococcosis (AE)**

A selection of drugs and potential drug candidates for the treatment of AE is provided in Table 2. Cyclosporin A, contrary to what was found in CE, lacked anti-parasitic activity against *E. multilocularis* infection in experimentally infected mice (Liance et al. 1992). Doxorubicin, or hydroxydaunorubicin, a DNA-interacting drug used widely in the treatment of a wide range of cancers (Lauchbury and Haboubi, 1993), was bound to polyisohexylcyanoacrylate nanoparticles (a colloidal biodegradable drug carrier) and applied in *E. multilocularis*-infected mice, resulting in the reduction of the development of the parasite in the liver and a reduced viability of the metacestode. Free doxorubicin or unbound nanoparticles had no antiparasitic activity (Liance et al. 1993). Animal experimentation in rodents demonstrated parasitostatic effects of mytomycin C, piperazine and quinolone derivatives, alkylaminoethers and propargyl alcohols, either at a lower level or comparable to benzimidazoles (reviewed in Siles-Lucas and Hemphill, 2002). The efficacy of praziquantel was inadequate (Marchiondo et al. 1994), although showing some effects on protoscoleces *in vitro* (Taylor and Morris, 1988). Also, the treatment of *E. multilocularis*-infected mice with alpha-difluoromethylornithine was not successful (Miyaji et al. 1993).

More recently, studies on chemotherapeutically interesting compounds have employed *in vitro* cultured parasites. Nitazoxanide, a broad-spectrum anthelmintic also used for treatment against enteric bacteria, *Giardia* and *Cryptosporidium* (Hemphill et al. 2006), was identified as a compound inducing significant distortion of the germinal layer *in vitro*, and nitazoxanide-treated *E. multilocularis* metacestodes were non-viable when introduced into susceptible mice (Stettler et al. 2003). Reuter et al. (2006) investigated the *in vitro* efficacy of a series of compounds against *E. multilocularis* metacestodes, including albendazole, artemether, caspofungin, itraconazole, ivermectin, methazol, miltefosine, nitazoxanide, rifampicin and trimethoprim/sulfamethoxazole. They found that albendazole, itraconazole, methazol and nitazoxanide effectively destroyed parasite vesicles *in vitro*. However, after drug discontinuation, re-growth of vesicles occurred, indicating a parasitostatic effect only. Combination treatment with albendazole/nitazoxanide at concentrations between 1 and 10 μg/ml for 3 weeks yielded no re-growth of parasites during 8 months of
Table 2. A selection of drug candidates against alveolar echinococcosis caused by *Echinococcus multilocularis*

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>In vitro</em> activity</th>
<th><em>In vivo</em> activity</th>
<th>Activity in humans</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporin A</td>
<td>Not assessed</td>
<td>No – rodents</td>
<td>Not assessed</td>
<td>Liance <em>et al.</em> 1992</td>
</tr>
<tr>
<td>Doxorubicin and colloidal carrier</td>
<td>Not assessed</td>
<td>Yes – rodents</td>
<td>Not assessed</td>
<td>Liance <em>et al.</em> 1993</td>
</tr>
<tr>
<td>Mitomycin C, piperazine and derivates, alkylamineothers, propargylic alcohols</td>
<td>Not assessed</td>
<td>Yes – rodents</td>
<td>Not assessed</td>
<td>Rev. in Siles-Lucas and Hemphill, 2002</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>Yes – protoscoleces</td>
<td>No – rodents</td>
<td>Not assessed</td>
<td>Taylor and Morris, 1988; Marchiondo <em>et al.</em> 1994</td>
</tr>
<tr>
<td>Mebendazole, albendazole*</td>
<td>Yes – metacestodes, protoscoleces</td>
<td>Yes – rodents</td>
<td>Yes</td>
<td>Rev. in Siles-Lucas and Hemphill, 2002</td>
</tr>
<tr>
<td>Flubendazole</td>
<td>Not assessed</td>
<td>Yes – rodents</td>
<td>No</td>
<td>Rev. in Siles-Lucas and Hemphill, 2002</td>
</tr>
<tr>
<td>Itraconazole*, methiazole*</td>
<td>Yes – cysts</td>
<td>Not assessed</td>
<td>Not assessed</td>
<td>Miyaji <em>et al.</em> 1993</td>
</tr>
<tr>
<td><em>α</em>-difluoromethyl-ornithine</td>
<td>Not assessed</td>
<td>No – rodents</td>
<td>Not assessed</td>
<td>Stettler <em>et al.</em> 2003, 2004; Reuter <em>et al.</em> 2006; Kern <em>et al.</em> 2008</td>
</tr>
<tr>
<td>Nitazoxanide* or nitazoxanide + albendazole</td>
<td>Yes – metacestodes</td>
<td>Yes – rodents</td>
<td>Only case reports. Nitazoxanide alone not effective. Some limited activity in combination with albendazole. Needs more studies</td>
<td></td>
</tr>
<tr>
<td>Artemether, caspofungin, ivermectin, miltefosine, rifampicin, trimethoprim/sulfamethoxazole</td>
<td>No – metacestodes</td>
<td>Not assessed</td>
<td>Not assessed</td>
<td>Reuter <em>et al.</em> 2006</td>
</tr>
<tr>
<td>Amphotericin B desoxycholate*</td>
<td>Yes – metacestodes</td>
<td>Not assessed</td>
<td>Yes (intravenous)</td>
<td>Reuter <em>et al.</em> 2003a, b</td>
</tr>
<tr>
<td>Genistein and derivates</td>
<td>Yes – metacestodes</td>
<td>Not assessed</td>
<td>Not assessed</td>
<td>Naguleswaran <em>et al.</em> 2006</td>
</tr>
<tr>
<td>Pyridinyl imidazoles</td>
<td>Yes – metacestodes</td>
<td>Not assessed</td>
<td>Not assessed</td>
<td>Gelmedin <em>et al.</em> 2008</td>
</tr>
<tr>
<td>2-Methoxyestradiol</td>
<td>Yes – metacestodes</td>
<td>No – rodents</td>
<td>Not assessed</td>
<td>Spicher <em>et al.</em> 2008a</td>
</tr>
<tr>
<td>Dihydroartemisinin, artesunate</td>
<td>Yes – metacestodes</td>
<td>No – rodents</td>
<td>Not assessed</td>
<td>Spicher <em>et al.</em> 2008b</td>
</tr>
<tr>
<td>Isoprinosine and derivates</td>
<td>Yes – protoscoleces</td>
<td>Yes – rodents</td>
<td>Not assessed</td>
<td>Lawton <em>et al.</em> 2001</td>
</tr>
</tbody>
</table>

* Parasitostatic
drug discontinuation, and the subsequent evaluation in a bioassay in gerbils did also not result in viable parasite infections. In this respect, Stettler et al. (2004) showed that nitazoxanide, applied orally to *E. multilocularis* infected mice, either alone or in combination with ABZ, exhibited a profound antiparasitic efficacy, with the albendazole/nitazoxanide combination yielding the most promising outcome in terms of reducing parasite weight. The pharmacokinetic analysis of corresponding serum levels in mice showed that the application of albendazole in combination with nitazoxanide increased considerably the levels and the half-life of albendazole sulfoxide (Stettler et al. 2004). Therefore, the increased efficacy observed in mice could be the result of an increased availability of albendazole sulfoxide in mice receiving the combination treatment. Despite these promising results, neither nitazoxanide monotherapy nor nitazoxanide-albendazole combination therapies were highly effective in human patients suffering from AE (Kern et al. 2008).

Amphotericin B desoxycholate (cAMB), an antifungal compound, effectively inhibited the growth of *E. multilocularis* metacestodes, first *in vitro*, and subsequently in human patients *in vivo* (Reuter et al. 2003a,b). A major limitation of cAMB is its mode of administration (intra-venous), which makes it unsuitable for prolonged use, except for salvage treatment (Reuter et al. 2003b). Also, the action of cAMB is only parasitostatic and, since the drug is nephrotoxic, its widespread use is limited. Nevertheless, prolonged application of cAMB for months to years may be feasible in some cases, as side effects are mild and serious organ damage does not appear to occur (Reuter et al. 2003b).

*In vitro* studies on *E. multilocularis* metacestodes and *E. granulosus* protoscolecites have shown that genistein, representing a major component of soya and the most prominent isoflavonoid, as well as a number of genistein derivatives, are also highly effective against these parasites. The molecular basis of the efficacy of genistein and its derivative Rm6423 have not yet been elucidated, but these compounds could interfere in signalling, for instance, through an endogenous metabolite of oestrogen with both anti-angiogenic and anti-tumour effects, 2-methoxyestradiol (2-ME) (reviewed by Schumacher and Neuhaus, 2001) was shown to induce severe damage to *E. multilocularis* metacestodes *in vitro* in a dose-dependent manner (Spicher et al. 2008a). However, 2-ME-treatment of experimentally infected mice did not result in a reduction in parasite weight compared to the control, demonstrating that *in vitro* and *in vivo* situations are not always comparable. Best results were achieved with a treatment using a combination of 2-ME and albendazole, which lead to a reduction in parasite weight compared to albendazole treatment alone, but results did not show statistical significance (Spicher et al. 2008a). *In vitro* treatment of *E. multilocularis* and *E. granulosus* larval stages with the antimalarials dihydroartemisinin and artesunate exhibited promising results, while 6 weeks of *in vivo* treatment of mice with infected *E. multilocularis* metacestodes had no effect. Again, combination treatments of both drugs with albendazole led to a substantial but statistically not significant reduction in parasite weight compared to results with albendazole alone (Spicher et al. 2008b).

The *in vitro* effect of isorprinosine and its derivatives has also been demonstrated against protoscolecites of *E. multilocularis* (Lawton et al. 2001).

**Relevance of *Echinococcus* metacestode screening models for other cestodes and trematodes**

Numerous studies have demonstrated that many of those drugs that were active in *E. granulosus* drug screening models were also of significant relevance for *E. multilocularis* and *vice versa* (see above). In addition, there is ample evidence that most compounds with good *in vitro* and *in vivo* efficacy against *Echinococcus* metacestodes are also of relevance for combatting infections by other cestode larval stages. For instance, cysticerci of *T. taeniaformis* were highly sensitive to praziquantel (Becker et al. 1981), and the same applied to *T. solium* and *T.pisiformis* (Garcia-Dominguez et al. 1991; Martinez Zedillo et al. 1992). Albendazole and mebendazole (or modified derivatives) were also active against *T. taeniaformis* cysticerci in experimentally infected mice (Verheyen et al. 1978; Jain et al. 1989). To date, albendazole and praziquantel, taken as short-term treatments (8–15 days) are the main drugs of choice for chemotherapeutic treatment of neurocysticercosis in humans (Garcia et al. 2003; Shandera and Kass, 2006). A recent *in vitro* study on *T. crassiceps* indicates that a combination of nitazoxanide and albendazole could be used for the treatment of cysticercosis infections (Palomares-Alonso et al. 2007), but *in vivo* evidence is still lacking. The effects of other benzimidazoles, such as flubendazole, were evaluated in *T. solium*-infected swine, with promising results (Tellez-Giron et al. 1981) that were subsequently confirmed in human patients (Tellez-Giron, 1984).

The activity of benzimidazole derivatives against *Hymenolepis* larvae was evaluated in the intermediate
host Tribolium confusum, demonstrating clear effects with regard to larval development (Novak and Blackburn, 1985). However, in the rodent model it was shown that benzimidazole drugs were only effective against H. nana oncosphere infection, but already developed cysticercoids were difficult to cure (Gupta et al. 1981; Maki and Yanagisawa, 1985), and neither flubendazole nor thiabendazole could clear H. nana cysticercoid infections in mice. Mebendazole and a series of modified benzimidazole derivatives were effective against H. nana and H. diminuta cysticercoids (Dubey et al. 1985).

In vitro studies on the effects of exposure of Mesocestoides corti tetrathyridia to anti-parasitic drugs revealed that liposomized praziquantel and albendazole had a deleterious effect on the parasite morphology and development (Hrckova et al. 1998; Britos et al. 2000; Saldana et al. 2001). The efficacy and mode of action of praziquantel were studied in the mouse model, showing that application of praziquantel had an adverse effect on the tetrathyridia burden in the liver and peritoneum (Hrckova and Velebny, 1995, 1997). Following the oral administration of mebendazole to M. corti-infected mice, a parasiticidal effect was observed (Heath et al. 1975; Eckert and Pohlenz, 1976). This is in contrast to other cestode larvae, against which mebendazole exerts a parasitostatic effect only. Deleterious actions of other drugs on Mesocestoides tetrathyridia, including cyclosporin A (Chappell et al. 1989) and albendazole (Terena et al. 1998), have also been reported.

Potentially, the Echinococcus screening models could also have some, albeit limited, relevance for a number of trematode species, including Schistosoma japonicum, S. mansoni, Chlonorchis sinensis, Fasciola hepatica and Opisthorchis viverrini. For instance, praziquantel is the drug of choice for the treatment of schistosomiasis (King, 2007). The drug is highly effective against the adult worm (as for cestodes), but has only a minor activity against the larval schistosomula. Another class of drugs that show similarities between Echinococcus and trematodes are the artemisinins and synthetic trioxolanes. These antimalarial drugs possess a broad spectrum of activity against trematodes, causing profound damage in vitro and substantially reducing the worm burden in experimentally infected mice (reviewed by Keiser and Utzinger, 2007 a, b). Echinococcus metacestodes were also susceptible to artesunate and dihydroartemisinin in vitro, while the schistosomula were found to be particularly susceptible to arteether and artesunate (Utzinger et al. 2002; Spicher et al. 2008 b). The promising activity of mefloquine, another anti-malarial drug, in mice experimentally infected with S. japonicum and S. mansoni, has been reported (Keiser et al. 2009), and recent studies in our laboratory also revealed that mefloquine has a profound impact on in vitro cultured E. multilocularis metacestodes (A. Hemphill et al. unpublished observations).

On the other hand, albendazole and mebendazole, the main anti-echinococcal drugs, have no impact on S. mansoni (Schmidt, 1998). In contrast, flubendazole, a mebendazole-derivative, was active against S. mansoni in experimentally infected mice (Nessim et al. 2000; Williams et al. 2003), and another benzimidazole, triclabendazole, was active against Fasciola hepatica (Robinson et al. 2001). As for Echinococcus, F. hepatica was susceptible to nitazoxanide treatment in vitro, and clinical studies showed that nitazoxanide could be used for the treatment of fasciolasis in children and adults (reviewed in Hemphill et al. 2006).

WHERE TO GO FROM HERE?

As outlined in this review, considerable efforts have been undertaken to improve the therapeutic options for the treatment of CE and AE (reviewed in Vuitton, 2009). These efforts have largely concentrated on the establishment of procedures on how to manage the diseases and by setting up guidelines for treatment and classifications of disease status. Although most successful to a large extent, the current benzimidazole-based chemotherapy is far from optimal and, owing to the limited efficacy of this class of compounds, their side-effects and their costs, alternative drugs or drugs that could be integrated into a combination treatment are clearly needed. Thus, considerably more input and support is needed from academic institutions as well as pharmaceutical and biotechnological industries and governmental agencies to provide solutions for these neglected diseases. A recent survey on the financial resources going into research and development funding in 2007 has shown, that HIV/AIDS received 1-1 billion US dollars, malaria and tuberculosis obtained over 400 million US dollars each, and kinetoplastid diseases and diarrhoeal diseases were granted over 125 and 114 million dollars each. On the other hand, helminth infections as a whole, encompassing nematodes, cestodes and trematodes, received only 51-6 million dollars (Voelker, 2009).

But finances alone will not provide novel possibilities. Until recently, Echinococcus drug discovery has been based on rather anecdotal reports, where a limited number of drugs belonging to a certain compound class have been investigated. However, the Echinococcus drug discovery process has been lacking several important aspects that are compulsory for successfully identifying the best and most interesting compounds. First, we need to gain access to comprehensive compound libraries, and we need to be able to screen these libraries. This can only be done through the implementation of easy-to-handle and reliable medium-to-high-throughput in vitro assays that allow screening of larger numbers of
anti-parasitic drugs in an efficient manner. Preferentially, these assays do not rely on subjective microscopic evaluation, but on objective criteria, such as specific markers (e.g. enzyme activities) that indicate parasite viability/intactness or non-viability/damage, and which could be detected preferentially in an (at least) semi-automated system. The development of such assays is ongoing, but should be intensified.

Secondly, large-scale drug screening activities are only possible if sufficient numbers of parasite organisms can be generated in vitro. This prerequisite has been fulfilled due to the pioneering work of Brehm and co-workers (see Spiliotis and Brehm, 2008), who developed an E. multilocularis metacestode in vitro culture system that allows the generation of massive numbers of metacestodes out of a relatively small quantity of parasite tissue. This does not only enable researchers to carry out numerous drug-screening assays, but also provides the basis for biochemical studies, including the identification of drug targets for specific compounds by affinity chromatography and mass spectrometry-based sequencing.

Thirdly, it is important to have access to genomic and EST databases. In 2008, shotgun sequencing of the E. multilocularis genome was completed (http://www.sanger.ac.uk/Projects/Echinococcus/). This opens the door for increased use of in silico approaches for drug target identification, similar to the discovery of the anti-metacestodical activity of clarithromycin reported by Mathis et al. already in 2005. In addition, the availability of a genome database permits drug target identification by affinity chromatography of parasite extracts on drug-coupled matrices and subsequent protein identification by mass spectroscopy (Müller et al. 2008a,b).

Furthermore, a system is required to verify putative drug targets and investigate their functional role, preferentially by genetic means, such as over-expression or silencing of genes of interest. The basis for this was set in 2008, when a method for the long-term in vitro cultivation and proliferation of primary cells isolated from axenically grown E. multilocularis metacestodes was established by Spiliotis et al. (2008). Isolated E. multilocularis cells were transiently transfected with a plasmid carrying the gene coding for the cyanofluorescent-protein (CFP), and the corresponding gene product was expressed and detected by Western blot analysis. When co-cultured with hepatocytes, cultured E. multilocularis cells form aggregates, and eventually undergo complete in vitro regeneration of metacestode vesicles. Prospectively, this could well lead to the development of transgenic larval stages, and even adult E. multilocularis worms, and genetic tools can be exploited to elucidate the exact functional role of putative drug targets in different stages of development (reviewed in Brhem and Spiliotis, 2008a).

Finally, the in vitro activity of a compound that exhibits outstanding performance can be verified in vivo, initially preferentially in a relevant small laboratory animal model. The murine or gerbil models for primary and secondary AE (Stettler et al. 2004; Reuter et al. 2006) represent reliable tools for such in vivo studies.

CONCLUSIONS

Approximately 2 billion helminth infections occur in humans worldwide, and these involving the larval stages of the three cestodes E. multilocularis, E. granulosus and T. solium are among the most serious and life-threatening ones (Brehm et al. 2006). From a practical point of view, the E. multilocularis model, in contrast to other cestodes, clearly fulfills the criteria that would allow for intensified drug-screening processes. It displays advantages such as rapid growth and proliferation in vitro, access to comprehensive genomic information and EST-databases, the possibility to maintain parasite isolates routinely in laboratory mice, and to verify in vitro results in a relevant in vivo model. Currently, the monitoring of therapy effectiveness in humans is based methods based on PET scan using 18F-deoxyglucose (Reuter et al. 2004). After optimization, such methods could be used for the follow-up of suitable drug candidates in mice or gerbils thereby further reducing the number of animals per study. Compounds that not only act parasitostatic but also parasitocidal against Echinococcus in vivo have not been discovered to date, but it is conceivable that such compounds exist, and that they would be a very useful addition to the arsenal of anti-parasitic drugs against cestodes and trematodes.

ACKNOWLEDGEMENTS

We apologize to those authors whose contributions could not be cited in this paper. This work was supported through the National Science Foundation (31-111780), the Novartis Research Foundation, Helvetia Sana Foundation, and the Swiss Life Jubiläumsstiftung. JM is a recipient of a research fellowship provided by Novartis Animal Health, and BS has been supported by the Karl Enigk Stiftung.

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Echinococcus metacestodes as laboratory models


