

REVIEW ARTICLE

Innovative chemotherapeutical treatment options for alveolar and cystic echinococcosis

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SUMMARY

Echinococcus granulosus and *Echinococcus multilocularis* are cestode parasites, of which the metacestode (larval) stages cause the diseases cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively. Albendazole and mebendazole are presently used for chemotherapeutical treatment. However, these benzimidazoles do not appear to be parasitocidal *in vivo* against AE. In addition, failures in drug treatments as well as the occurrence of side-effects have been reported. New drugs are needed to cure AE and CE, which are considered to be neglected diseases. Strategies currently being implemented to identify novel chemotherapeutical treatment options include (i) conventional primary *in vitro* testing of broad-spectrum anti-infective drugs, either in parallel with, or followed by, animal experimentation; (ii) studies of drugs which interfere with the proliferation of cancer cells and of *Echinococcus* metacestodes; (iii) exploitation of the similarities between the parasite and mammalian signalling machineries, with a special focus on targeting specific signalling receptors; (iv) *in silico* approaches, employing the current *Echinococcus* genomic database information to search for suitable targets for compounds with known modes of action. In the present article, we review the efforts toward obtaining better anti-parasitic compounds which have been undertaken to improve chemotherapeutical treatment of echinococcosis, and summarize the achievements in the field of host-parasite interactions which may also lead to new immuno-therapeutical options.

Key words: cystic echinococcosis (CE), alveolar echinococcosis (AE), chemotherapy, immunotherapy, signalling, drugs, host-parasite interactions.

ECHINOCOCCUS AND ECHINOCOCCOSIS

Echinococcosis, caused by the larval stages of the cestode parasite of the genus *Echinococcus*, is a life-threatening disease of serious public health and economic concern of global proportion (Torgerson, 2003; Eckert and Deplazes, 2004). Four distinct species within the genus *Echinococcus* have been identified i.e. *Echinococcus multilocularis*, *E. granulosus*, *E. vogeli* and *E. oligarthrus* (Thompson, 1986). All *Echinococcus* species are potentially zoonotic, but only 2 are of significant medical importance in humans: *E. multilocularis* (small fox tapeworm) being the most pathogenic parasite, and *E. granulosus* (dog tapeworm) being the commonest (Rausch, 1995; McManus *et al.* 2003).

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E. multilocularis infection causes alveolar echinococcosis (AE) in intermediate hosts (rodents) and humans, and is restricted to the northern hemisphere. In contrast, *E. granulosus*, the causative agent of cystic echinococcosis (CE) that occurs worldwide (Schantz *et al.* 1995). The habitat of the adult worms is the small intestine of their respective definitive host (canids for *E. granulosus*, and canids and felids for *E. multilocularis*), where sexual reproduction and subsequent egg production take place. Eggs released in the faeces into the environment, where they are accidentally ingested by suitable intermediate hosts, such as small rodents for *E. multilocularis*, and mainly livestock for *E. granulosus*. Humans represent an aberrant intermediate host, acquiring the disease through the accidental ingestion of eggs, with serious consequences. Each egg contains an oncosphere (=first larval stage), which actively penetrates the intestinal lining and is transported *via* the blood and lymph to the sites of predilection/establishment. Affected organs in humans are mainly the liver for *E. multilocularis* and the liver and

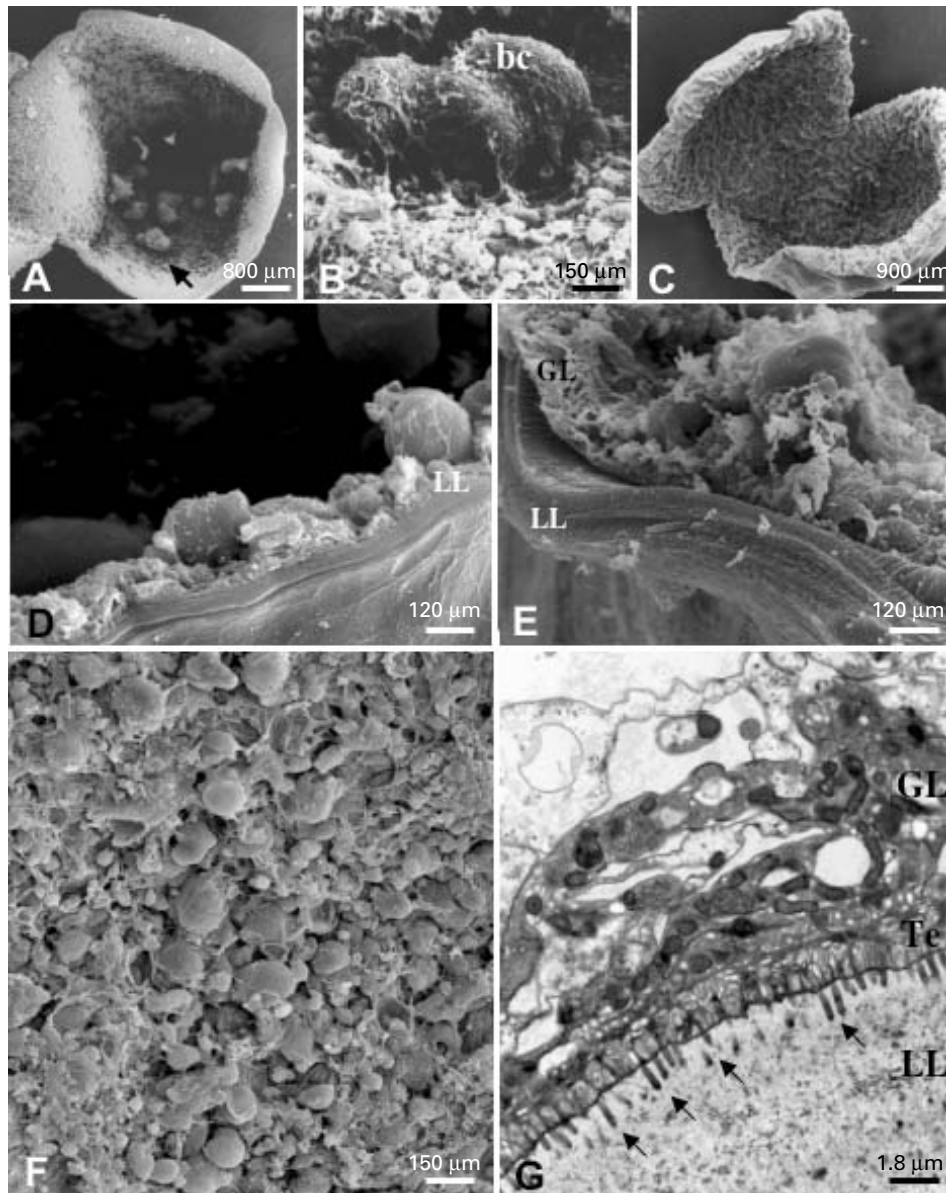


Fig. 1. Morphology and ultrastructure of *Echinococcus* metacestodes. (A) *E. multilocularis* metacestode, cut open to view the germinal layer with developing brood capsules (arrow); (B) SEM of brood capsules (bc) containing developing protoscolexes; (C) *E. granulosus* metacestode cultured *in vitro*; (D) SEM showing laminated layer and germinal layer of *E. multilocularis* metacestode; (E) SEM of laminated layer and germinal layer of *E. granulosus* metacestode; (F) SEM of germinal layer-associated tissue of *E. multilocularis*; (G) TEM of *E. granulosus* metacestodes showing laminated layer, tegument and germinal layer. GL = germinal layer, LL = laminated layer, Te = tegument, arrows in (G) point towards microtriches.

lung in the case of *E. granulosus*. In these sites, the oncospheres develop into metacestodes (=second larval stage). Within these metacestodes, *via* asexual reproduction, brood capsules and protoscolexes form in natural intermediate hosts. If the metacestode in/ from an infected intermediate host is ingested by a suitable definitive host, the life-cycle is concluded. Protoscolex development in humans has only rarely been described (Gottstein and Hemphill, 1997).

Metacestodes represent the disease-causing stage. They are fluid-filled cysts with cellular and acellular compartments (Fig. 1). The outer, acellular surface

of the metacestode is formed by the laminated layer, a carbohydrate-rich structure, synthesized by the parasite, which, in terms of thickness, is much more prominent in *E. granulosus* than *E. multilocularis* metacestodes (Gottstein and Hemphill, 1997). In addition, *E. granulosus* metacestodes are surrounded by a very prominent host-derived fibrous capsule, the adventitial layer, which is composed of host connective tissue. The laminated layer plays a crucial role in the survival strategy of the parasite by modulating immunological and physiological reactions of the host (Gottstein *et al.* 2002; reviewed

in Siles-Lucas and Hemphill, 2002). The actual larval tissue is formed by the germinal layer, the distal part of which consists of the tegument which is directly associated with the inner surface of the laminated layer. The tegument is characterized by microvilli-like extensions termed microtriches, which protrude well into the matrix of the laminated layer and increase the resorbing surface of the parasite. In addition, the germinal layer contains highly differentiated cell types, including connective tissue, muscle cells, glycogen storage cells and many undifferentiated cells (Eckert *et al.* 1983).

Typically, the fully mature *E. granulosus* metacestode (i.e. hydatid cyst) is a single-chambered or septated but usually unilocular cyst, which displays expansive growth, causing a compression of neighbouring tissue and resulting in organ dysfunction and disease (Kern, 2003, 2006). In *E. multilocularis* infection, the metacestode represents a multi-vesicular structure which reproduces (also asexually) by exogenous formation and budding of daughter vesicles, which resembles progressive tumour-like growth (Ohbayashi *et al.* 1971; Ali-Kahn *et al.* 1983). This growth leads to the a large and heterogeneous mass of the parasite characterized by mostly peripheral actively proliferating areas and, in many cases, centrally located necrotic tissue. Metastases can occur in other organs following the release of germinal layer cells into the blood or lymph system (Ali-Kahn *et al.* 1983; Eckert *et al.* 1983; Mehlhorn *et al.* 1983).

LIMITATIONS OF CURRENT CHEMOTHERAPEUTICAL TREATMENT

Traditionally, the treatment of echinococcosis relies primarily on surgery and/or chemotherapy, and the treatment strategy depends largely on different factors, such as metacestode size and location, viability status, the interaction between the expanding parasite and the adjacent host tissue, complicating bacterial or fungal infection and potential complications related to cyst rupture and spillage of protoscoleces (Kern, 2003, 2006).

In CE, radical resection of the cyst mass represents the traditional treatment strategy and is, in many instances, accompanied by chemotherapy prior to and following surgery. Protoscolicidal substances are often applied, since there is a risk of spillage of cyst fluid containing protoscoleces, which can lead to metastases (Stey and Jost, 1993; Pawloski, 1997; Kern, 2003, 2006). PAIR (puncture, aspiration, injection, re-aspiration) is a technique that was introduced in the mid-eighties, and includes percutaneous puncture of the cysts under ultrasonic guidance, aspiration of substantial amounts of cyst fluid, injection of protoscolicidal substance (e.g. 95% ethanol), and re-aspiration of the fluid cyst content after 15–20 min. Although

the efficacy and potential risks have not been fully evaluated, and more long-term studies are needed, PAIR has been used in several hundred patients (Eckert and Deplazes, 2004; Brunetti *et al.* 2004). For inoperable cases, chemotherapy applying benzimidazoles is the only option. There are over 2000 well-documented inoperable cases of CE, which have undergone treatment with benzimidazoles. When evaluated up to 12 months after initiation of chemotherapy, cysts disappeared in 30% of patients, cysts degenerated in 50–70%, indicating improvement, and in 20–30% of patients *E. granulosus* metacestodes did not respond to chemotherapy (Pawloski *et al.* 2001). Praziquantel (PZQ), a heterocyclic pyrazinoisoquinoline derivative has been proposed for use together with benzimidazoles in CE patients. PZQ exhibits a high efficacy against protoscoleces and metacestodes in animal experiments (Urrea-Paris *et al.* 1999, 2001), and the combined treatment with ABZ and PZQ given during the month prior to surgery has been shown to increase the number of human patients with non-viable protoscoleces as compared to therapy with ABZ alone (Cobo *et al.* 1998).

For the treatment of AE, surgery, if applicable, is accompanied by chemotherapy using benzimidazoles, which should be maintained for at least 2 years following surgery, and the monitoring of patients should be continued for 10 years (Pawloski *et al.* 2001). Inoperable AE cases must undergo long-term, often life-long, chemotherapy with albendazole (ABZ) and/or mebendazole (MBZ) (Reuter *et al.* 2000, 2004). Extensive animal experimentation and observations in human patients suffering from AE have demonstrated that ABZ and MBZ exhibit a parasitostatic rather than a parasitocidal effect (El-On, 2002; Reuter *et al.* 2004). Thus, benzimidazoles only prevent *E. multilocularis* 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% (Ammann and Eckert, 1995; Eckert and Deplazes, 2004). Spontaneous cure of AE, leading to calcified lesions is possible, but it is not known how frequently calcification occurs (reviewed by Gottstein and Hemphill, 1997; Vuitton *et al.* 2006). Large surveys in endemic areas have shown that the number of patients with established AE is considerably lower than the number of seropositive humans exposed to the eggs of *E. multilocularis* (see Rausch *et al.* 1987; Bresson-Hadni *et al.* 1994; Bartholomot *et al.* 2002). This information suggests that immunity in humans is capable of killing the parasite after infection, which could facilitate the development of immunotherapeutical tools.

The clinical application of benzimidazoles for the treatment of AE and CE was accompanied by studies

employing animal models, focusing on comparing the activities of different benzimidazole derivatives, and on different formulations and modes of application. The efficacies of oral administration were demonstrated to be dependent on the duration of treatment and the age of the parasite. Efficacy increases with the duration of treatment, but is decreased for infections which have persisted for long periods of time (over 6 months in mice) (Wangoo *et al.* 1987). Increasing doses produced better results, although clear parasitocidal effects were not achieved (Taylor *et al.* 1989), and observations consistent with drug resistance were described (Morris and Taylor, 1990). There are conflicting reports of the most suitable mode of administration of benzimidazoles. It was postulated that parenteral administration of benzimidazoles resulted in a higher efficacy than other routes in animals experimentally infected with *E. multilocularis* (reviewed by Siles-Lucas and Hemphill, 2002). Drug combinations, normally consisting of one benzimidazole and one or more other compound, were tested in order to obtain better treatment efficacies. For instance, synergistic effects were reported for combinations of ABZ with the dipeptide methyl ester Phe-Phe-OMe (Sarciron *et al.* 1997). In addition, novel formulations of benzimidazoles, either as pro-drugs (Walchshofer *et al.* 1990), liposome-entrapped compounds (Wen *et al.* 1996) or colloidal, intravenously injectable formulations (Rodrigues *et al.* 1995) were tested in rodents and showed enhanced efficacy at lower doses than the parental compounds. However, these studies have not yet been translated into clinical applications, with one exception: Chai *et al.* (2004) reported on improved efficacy of ABZ emulsion compared with ABZ tablets or capsules for the treatment of liver CE.

Adverse reactions against benzimidazoles under long-term chemotherapy include elevation of transaminases, proteinuria, hair loss, gastrointestinal disturbances, neurological symptoms (vertigo/dizziness), leukopaenia, headache, abnormal liver biopsy, abdominal pain, fever, urticaria, thrombocytopenia, allergic shock (due to cyst collapse and release of *E. granulosus* cyst fluid) and toxicity to bone marrow. A study comprised of 3282 echinococcosis patients treated with ABZ showed that most side-effects were associated with the gastrointestinal tract (Pawloski, 1997; Kern, 2003), but no fatal cases involving ABZ therapy have been described. In 3.8% of these cases, permanent discontinuation of treatment had to be undertaken. As suggested by animal experimentation (Horton, 1989, 1997), MBZ and ABZ may induce embryotoxic or teratogenic effects, and it is recommended that these drugs are not used for the treatment of pregnant women. Constant monitoring of drug levels in serum is suggested in order to prevent toxic reactions.

TOWARDS THE DEVELOPMENT OF IMMUNOTHERAPEUTICAL APPROACHES FOR THE TREATMENT OF ECHINOCOCCOSIS

Considerable effort has gone into elucidating the immunological basis of the host-parasite relationship during infection with *Echinococcus*. In both AE and CE, the initial immune responses during the primary phases of infection are characterized by a predominantly Th1-type response, whereas in the later stages of disease progression, the immune response switches to a Th2 polarized profile (Gottstein *et al.* 2006; Vuitton *et al.* 2006; Zhang and McManus, 2006). In addition, one of the hallmarks of most helminth infections is the occurrence of a profound immuno-modulation and -suppression (Maizels and Yazdanbakhsh, 2003). Hence, *E. granulosus* and *E. multilocularis* have a significant influence on the immune response in their hosts. For both *E. multilocularis* and *E. granulosus*, it has been shown that carbohydrate moieties or carbohydrate-rich fractions possess immunomodulatory properties (Dai *et al.* 2001; Dematteis *et al.* 2001; Walker *et al.* 2004a; reviewed by Baz *et al.* 2006). This information is in accordance with findings of studies investigating the involvement of glycans in immunomodulation during helminth infections (Maizels and Yazdanbakhsh, 2003), and the current challenge is to translate this knowledge into novel immuno-therapeutic applications.

Cystic echinococcosis

Some insights into the initial phases of CE have been obtained using experimental *E. granulosus* infection in sheep or mice (Baz *et al.* 2006; Zhang and McManus, 2006). The initial low-level Th1-biased immune response following the infection is accompanied by an infiltration of macrophages and eosinophils and the development of a weak humoral immune response. Conversely, established cysts in experimentally infected animals and in human patients are capable of modulating the immune response, resulting in a Th2-polarized profile exhibiting the production of IL-4, IL-5, IL-6 and IL-10 and balanced by Th1 cytokines, such as IFN- γ . Upon the death of an *E. granulosus* cyst either naturally or due to chemotherapy, levels of Th2 cytokines decrease rapidly and the immune response polarizes towards Th1; upon relapse, Th2 responses regenerate within a few weeks. This Th1-Th2 co-existence and inter-conversion during CE is likely to be mediated by *E. granulosus* antigens. Examples of antigens which bear epitopes which can induce both Th1 and Th2 responses are Eg2HSP70, EgA31 and Eg/Trp, (Ortona *et al.* 2003; Fraize *et al.* 2005). Another efficient strategy which contributes to the parasite survival strategy is the disruption of effector mechanisms of the host immune response. In a study of *E. granulosus*, Diaz and co-workers

showed that the activation of complement is inhibited by the binding of the host inhibitory Factor H to the hydatid cyst wall. The same authors investigated the consumption of complement components by molecular factors within the hydatid cysts (Diaz *et al.* 1999; Ferreira *et al.* 2000, 2001). In addition, T cell responses were shown to be modulated by the intensity of infection, as demonstrated in mice infected with different doses of *E. granulosus* protoscoleces (Dematteis *et al.* 2003). The generation of T cell lines from patients exhibiting different courses of disease (harbouring active, transitional or inactive cysts) and subsequent stimulation of these cells with hydatid fluid and/or a defined *E. granulosus* antigen (AgB) has also shown that Th1 cells contribute to the inactive stage of hydatid disease, whereas Th2 cells have been shown to be more important in the active and transitional stages (Rigano *et al.* 2004). Interestingly, patients undergoing chemotherapy with benzimidazoles exhibited a more pronounced Th1 cytokine profile, reflecting the regression of the metacestode (Rigano *et al.* 1995). Self cure of CE, leading to calcified cysts, is common in sheep and also in the human population in hyperendemic regions (MacPherson *et al.* 2004; Moro *et al.* 2005), and cytokines are likely to play a key role in these processes. Elucidating the factors involved in cyst calcification could lead to novel therapeutical approaches.

Alveolar echinococcosis (AE)

In accordance with *E. granulosus* infection in humans, Th1 responses dominate the early stages of *E. multilocularis* infection, with the immune response switching to Th2 at the chronic stage of disease (Zhang and McManus, 2006). Antibody levels are low initially, but levels of IgG1 and IgG3 increase during infection. In human patients, a strong cellular immune response is a characteristic hallmark, resulting in granulomatous infiltrate surrounding the parasite lesions (Vuitton *et al.* 1989; Grenard *et al.* 2001). In patients with abortive or dead lesions, the cells contributing to this granuloma formation are mostly macrophages, myofibroblasts and a large number of CD4+ T cells, and in patients exhibiting a progressive clinical course of AE, there is an increased number of CD8+ T cells (Manfras *et al.* 2002). This corresponds to findings in mice, which have shown that *E. multilocularis* metacestodes exhibit a considerably increased growth in nude, TCR- β (-/-) and MHC-II (-/-) mice compared with wild type C57BL/6 mice, with the T cell-deficient mice dying 2 months after infection (Dai *et al.* 2004). Thus, CD4+ $\alpha\beta$ + T cells play a growth-limiting role in *E. multilocularis* infection.

A further hallmark of AE is the development of fibrosis surrounding the parasite. In human patients, fibrotic processes are frequently observed, even

distant from the lesions caused by the parasite, suggesting a major role for cytokines in collagen synthesis and cross-linking (Vuitton *et al.* 2006). There are indications that fibrotic processes might be directed by the parasite itself. A parasite trans-glutaminase has been shown to be strongly expressed in and at the border of metacestodes, and this enzyme was capable of cross-linking human collagen *in vitro* (Grenard *et al.* 2001). Fibrosis itself, in addition to the outer acellular laminated layer, could be responsible for keeping cytolytic immune cells away from the parasite tissue.

The observation that a strong cellular immunity renders mice less susceptible to disease and that Th1 type immune responses, as assessed by corresponding cytokines, may kill the parasite or prevent its development suggests that immuno-therapeutical tools could be developed to combat echinococcosis. This notion is supported by evidence that the stimulation of the immune system with Bacille Calmette Guerin (BCG) has been reported to exhibit a profound reductive effect on the size and dissemination of *E. multilocularis* metacestodes in rodents (Rau and Tanner, 1975). However, despite encouraging clinical results, there are no reports on the limited BCG-trials in human patients (Vuitton *et al.* 2006).

Another immuno-stimulating compound, Iso-prinosineTM, has been shown to have a good efficacy against *E. granulosus* and *E. multilocularis* metacestodes and protoscoleces *in vitro* as well as in mice and jirds (Sarciron *et al.* 1992, 1993, 1995; Lawton *et al.* 2001). The drug has been applied in patients with AE, and has shown a regression of parasite lesions in 2 patients (reviewed by Vuitton *et al.* 2006). This compound acts through stimulating cellular immunity, increasing IL-1, IL-2 and IFN- γ -production, and inhibiting Th2 type immune responses, including IgE-dependent reactions.

The pre-treatment of mice with IL-12 has been shown to efficiently limit the development of parasite lesions, resulting in aborted metacestode vesicles surrounded by infiltrative cells and fibrosis (Emery *et al.* 1998). However, IL-12 treatment has not been evaluated as a therapeutic agent in human AE patients because of potential side-effects.

Liance *et al.* (1998) reported that the *in vivo* treatment of mice with a low dose (1 μ g, twice a week for 3 weeks) of IFN- γ decreased metacestode growth and liver fibrogenesis. The effect was dose-dependent, as the treatment with a higher dose (5 μ g, twice a week for 3 weeks) increased the number of metacestodes in the liver. Two case reports documented the clinical efficacy of IFN- γ -treatment. One patient suffering from severe side-effects caused by ABZ-therapy was treated with IFN- γ during a 3-day period once a month, and this treatment prevented disease progression but did not alter the Th2-dominated immune response against this parasite

(Jenne *et al.* 1998). Another patient whose lesions were growing, despite mebendazole therapy, was treated with a combination of IFN- γ and mebendazole, which halted the progression of disease as revealed in a 1-year follow-up period (Schmid *et al.* 1995).

The treatment of mice with IFN- α -2a has been shown to largely prevent the formation of hepatic lesions in infected mice, with an inhibition of IL-10, IL-6 and IL-13 antigen-induced secretion in spleen cell cultures (Godot *et al.* 2003). One patient with AE and chronic hepatitis C was treated effectively with IFN- α -2a, suggesting that this cytokine limited parasite growth and reversed the cytokine profile to Th1 (Harraga *et al.* 1999). Thus, IFN- α -2a seems to be the most promising candidate for, for instance, further clinical investigation, because it is already widely used for treating chronic viral infections. However, more studies are required to assess the ambiguous role of this cytokine.

SEARCHING FOR NOVEL CHEMOTHERAPEUTICAL OPTIONS

As discussed, novel and improved therapeutical tools are needed in order to optimize treatment of CE and AE. Both *in vitro* and *in vivo* laboratory models have been used for drug evaluation (reviewed by Siles-Lucas and Hemphill, 2002). Unfortunately, besides the initial development of benzimidazoles, the pharmaceutical industry has not expressed a keen interest in supporting the discovery of novel treatment options. Therefore, AE and CE must be regarded as neglected diseases.

Historically, the primary assessment of anti-echinococcal drug candidates has often been performed in rodents (mice or gerbils), which has led to the extensive use of animal experimentation. Subsequently, the *in vitro* culture of *Echinococcus* metacestodes has proven to be a suitable tool for the primary assessment of parasite susceptibility to certain compounds, with a focus on broad-spectrum anti-infective agents, and also represents an ideal model system for studies on drug uptake and associated metabolic changes imposed upon the parasite (Hemphill *et al.* 2002). More recently, optimized *in vitro* culture conditions (Spiliotis *et al.* 2004) have allowed the dissection of the molecular nature of the signalling machinery within *E. multilocularis* metacestodes required for communication at the host-parasite interface (reviewed by Brehm *et al.* 2006). These studies have provided extensive information on novel potential drug targets associated with the parasite signalling network. Although the availability of genomics and related technologies provides avenues for the application of modern approaches, the current genomic and expressed sequence tag (EST) information on *Echinococcus* is still limited. Nonetheless, the generation of more

EST data is underway (see <http://www.sanger.ac.uk/Projects/Echinococcus/>) and will be an extremely valuable resource for gene expression studies.

The following strategies are currently being followed in order to identify novel alternative chemotherapeutical treatment options: (i) conventional primary *in vitro* testing of broad-spectrum anti-infective drugs, either in parallel with, or followed by, animal experimentation; (ii) studies on drugs which interfere with the uncontrolled proliferation of cancer cells and affect the viability of *Echinococcus* metacestodes and protoscoleces; (iii) exploitation of the similarities between the parasite and mammalian signalling machineries, with a special focus on targeting specific signalling receptors; (iv) *in silico* approaches, employing the current *Echinococcus* genomic database information to search for suitable targets for compounds of known mode of action.

(i) Chemotherapeutical activities of anti-infective drugs

In vitro chemotherapy studies of CE have mostly, but not exclusively, focused on protoscoleces, since these are easily cultured, and their differentiation into metacestodes is a time-consuming process that can easily take 4–6 months (Walker *et al.* 2004b). Experimental prophylactic therapy of *E. granulosus* protoscoleces was carried out as a model that would mimic spillage during surgery, by treating protoscoleces with PZQ (Urrea-Paris *et al.* 2001) or a combination of PZQ and ABZ (Casado *et al.* 2001) prior to injection into mice. Respective findings are of substantial clinical relevance. Other promising compounds with *in vitro* protoscolicidal actions were cetrimide (Frayha *et al.* 1981) and the ionophore monensin (Rogan and Richards, 1986), but these drugs were found to be rather ineffective against metacestodes. Levamisole and ivermectin, which are classically nematocidal, were shown to exhibit *in vitro* activities similar to benzimidazoles (Martinez *et al.* 1999). Against *E. granulosus* infection in rodents, a combination of fenbendazol and netobimin (Garcia-Llamarez *et al.* 1997) showed synergistic effects, allowing the administration of lower drug dosages. Oxfendazole, like ABZ, 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 ABZ, but does not require daily uptake of the drug because of its prolonged bioavailability (Blanton *et al.* 1998; Dueger *et al.* 1999). Xiao *et al.* (1995) studied the effects of several drugs on enzymes involved in carbohydrate metabolism and found that some of the corresponding host enzymes

were not affected, thus identifying novel potential drug targets. Cyclosporin A, employed mainly as an immunosuppressant during the management of organ transplants, also exhibits anti-echinococcal activity. While the administration of cyclosporin A in 5 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; 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).

In rodents infected with *E. multilocularis* metacestodes, mytomicin C, piperazine and quinolone derivatives, alkylaminoethers and propargylic alcohols exhibited parasitostatic effects, at either a lower or comparable level as benzimidazoles (reviewed by Siles-Lucas and Hemphill, 2002). PZQ has been used for the treatment of AE, but experimental data in animals have shown that the efficacy of PZQ against *E. multilocularis* metacestodes was inadequate (Marchiondo *et al.* 1994). Also, the treatment of *E. multilocularis*-infected mice with alpha-difluoromethylornithine was not successful (Miyaji *et al.* 1993). In contrast to its use against CE, cyclosporin A did not have any anti-parasitic activity against *E. multilocularis* infection in experimentally infected mice, and its immunosuppressive activity was shown to be more effective than its parasitostatic effect (Liance *et al.* 1992).

Nitazoxanide (NTZ), a broad-spectrum anthelmintic also used for treatment against enteric bacteria, *Giardia* and *Cryptosporidium* (cf. Hemphill *et al.* 2006), was identified as a compound inducing significant distortion of the germinal layer *in vitro*, and NTZ-treated *E. multilocularis* metacestodes were non-viable when introduced into susceptible mice (Stettler *et al.* 2003). NTZ was also found to induce severe damage to *E. granulosus* protoscoleces and the germinal layer of *in vitro*-cultured *E. granulosus* metacestodes (Walker *et al.* 2004b). NTZ represents the parent compound of a class of drugs named thiazolides, which include modified variants of NTZ (Hemphill *et al.* 2006; Esposito *et al.* 2007). *In vitro* studies of *E. multilocularis* and *E. granulosus* employing NTZ-derivatives have shown that metacestodical and protoscolicidal activities of this class of drugs strongly depend on the presence of the nitro-thiazole moiety, suggesting that this nitro-group is instrumental for the activity of thiazolides (Naguleswaran and Hemphill, unpublished findings). These promising results indicate the potential of NTZ and possibly other

thiazolides as novel anti-echinococcal compounds (Craig, 2003).

Recently, Reuter *et al.* (2006) investigated the efficacy of a series of compounds against *E. multilocularis* metacestodes, including ABZ, artemether, caspofungin, itraconazole (ITZ), ivermectin, methiazole (MTZ), miltefosine, NTZ, rifampicin and trimethoprim/sulfamethoxazole. They found that ABZ, ITZ, MTZ and NTZ effectively destroyed parasite vesicles *in vitro*. However, after drug discontinuation, re-growth of vesicles occurred, indicating a parasitostatic effect only. Combination treatment with ABZ/NTZ at concentrations between 1 and 10 µg/ml for 3 weeks yielded no re-growth of parasites during 8 months of drug discontinuation, and the subsequent evaluation in a bioassay in gerbils did also not result in viable parasite infections. These results indicated that combined ABZ/NTZ treatment exhibits a parasitocidal effect. In this respect, Stettler *et al.* (2004) have shown that NTZ, applied orally to *E. multilocularis*-infected mice, either alone or in combination with ABZ, exhibited a profound anti-parasitic efficacy, with the ABZ/NTZ combination yielding the most promising outcome. Electron microscopical analysis of metacestode tissue obtained from treated mice suggested that the ABZ/NTZ combination exerted a synergistic effect. However, the pharmacokinetic analysis of corresponding serum levels in mice showed that the application of ABZ in combination with NTZ increased considerably the ABZSO-levels and also the half-life of ABZSO (Stettler *et al.* 2004). Therefore, the increased efficacy observed in mice could be the result of both direct effects of NTZ and ABZ metabolites and increased availability of ABZSO in mice receiving the combination treatment.

Amphotericin B desoxycholate (cAMB), an anti-fungal compound, was shown to effectively inhibit the growth of *E. multilocularis* metacestodes *in vitro* and in human patients *in vivo* (Reuter *et al.* 2003a,b). A major limitation of cAMB is its mode of administration (intravenous), which makes it unsuitable for prolonged use, except for salvage treatment (Reuter *et al.* 2003b). Also, the effect of cAMB is only parasitostatic and the drug is nephrotoxic, limiting its widespread use. 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).

(ii) Anti-cancer drugs and echinococcosis

There are a number of similarities between cancer cells and some parasites, particularly *Echinococcus* (reviewed by Klinkert and Heussler, 2006). Similarities include features, such as the essentially

unlimited proliferative capacity of protoscolecids/brood capsules, the potential to modulate the immune response, the secretion of proteolytic enzymes to reach their target sites or organs, and the formation of metastases. *E. multilocularis* metacystodes behave like malignant tumours, and there is an association between the uncontrolled proliferation and growth and the over-expression in metacystodes of a family of proteins named 14-3-3 (Siles-Lucas *et al.* 1998, 2001). 14-3-3 proteins are found in all eukaryotic cells and participate in protein kinase signalling pathways. They function as phosphoserine/phosphothreonine-binding modules and have an effect on phosphorylation-dependent events, such as DNA-damage checkpoints and prevention of apoptosis (reviewed by Siles-Lucas and Gottstein, 2003). Some 14-3-3 proteins have been found to be aberrantly expressed in tumour cells, acting either pro- or anti-tumorigenically. Indeed, when *Echinococcus* 14-3-3 sequences are aligned with other 14-3-3 isoforms of other organisms, those over-expressed in metacystodes can be grouped with the tumour-growth related zeta-isoforms (Siles-Lucas *et al.* 2001). This information suggests that certain anti-tumour agents could interfere with the growth of *Echinococcus* metacystodes.

Doxorubicin, or hydroxydaunorubicin, is a DNA-interacting drug used widely in the treatment of a wide range of cancers (Launchbury and Habboubi, 1993). The parasitocidal properties of doxorubicin bound to polyisohexylcyanoacrylate nanoparticles (a colloidal biodegradable drug carrier) were assessed against the metacystode of *E. multilocularis* (see Liance *et al.* 1993). A reduction of the development of the parasite in the liver and a reduced viability of the metacystode were observed in mice injected with 5 mg/kg body weight, but 7.5 mg/kg body weight did not appear more efficient. Free doxorubicin or unbound nanoparticles had no anti-parasitic activity (Liance *et al.* 1993).

Another class of anti-cancer agents with proven anti-parasitic activities are isoflavonoids. Isoflavonoids are substances formed by plant tissue in response to physiological stimuli, such as infectious agents, with reported anti-oxidant, -bacterial, -viral and -fungal activities (Dakora and Philipps, 1996). They are composed of a characteristic 15-carbon backbone ring structure connected by a heterocyclic pyrane (3-C) bridge (C6-C3-C6) (Reynaud *et al.* 2005), with the 2 aromatic rings generally containing a number of phenolic hydroxyl groups. Genistein, a major component of soya, is the most prominent isoflavonoid, and inhibits growth and metastasis of a number of cancer cell lines (breast, prostate, skin and colon) (Messina, 1999). Genistein also stimulates the synthesis of TGF- β , which itself inhibits cancer cell proliferation (Messina, 1999). Besides other targets, genistein acts on a number of signalling pathways by functioning

as a kinase inhibitor (tyrosine kinase, MAP kinase, ribosomal S6 kinase). Recent studies have shown that genistein is highly effective against *E. multilocularis* metacystodes *in vitro* (Naguleswaran *et al.* 2006). However, genistein has a disadvantage, in that it also exerts oestrogenic effects by binding to oestrogen receptor- β , which renders genistein unfavourable for long-term treatment. Binding to the oestrogen receptor- β has been proven to take place through the hydroxyl-group associated with the B-ring of the molecule. Therefore, a number of isoflavonoids that do not carry this hydroxyl-group have been tested *in vitro* and do not meet the steric requirements to bind to the oestrogen receptor-beta. One of these compounds, Rm6423, exhibits a pronounced anti-parasitic activity against *E. multilocularis* metacystodes as well as against *E. granulosus* metacystodes and protoscolecids (Naguleswaran *et al.* 2006). Moreover, an examination of culture medium revealed increased leakage of parasite proteins into the medium during treatment, and zymography demonstrated a loss of the activity of metalloproteases. The molecular basis of the efficacy of genistein and its derivative Rm6423 has not yet been elucidated, but these compounds could interfere in signalling, for instance, through an inhibition of the tyrosine kinase activity associated with the epidermal growth factor receptor identified in *E. multilocularis* (see Spiliotis *et al.* 2006).

An endogenous metabolite of oestrogen with both anti-angiogenic and anti-tumour effects, 2-methoxyestradiol (2-ME) (reviewed by Schumacher and Neuhaus, 2001) has been shown to down-regulate the pro-tumorigenic 14-3-3- ζ -isoform in a number of cancer cell types (Kumar *et al.* 2003). The pro-tumorigenic 14-3-3- ζ -isoform is also over-expressed in *Echinococcus* metacystodes (Siles-Lucas and Gottstein, 2003), and the exposure of 2-ME (2–10 μ M) to *E. multilocularis* metacystodes *in vitro* severely damaged them in a dose-dependent manner. In parallel, drug treatment also down-scaled 14-3-3 transcription compared with actin expression (Naguleswaran *et al.* unpublished findings). Although it is not known how 2-ME exerts its effects on *Echinococcus*, the mechanism of action of 2-ME in cancer cells has been attributed to an interference with microtubule stability and a dysregulation of the hypoxia-inducible factor (Klauber *et al.* 1997; Majeesh *et al.* 2003) inducing cancer cells to undergo apoptosis *via* extrinsic and intrinsic pathways.

(iii) The *Echinococcus* signalling machinery as a novel drug target

More recently, the excellent work of Brehm *et al.* (2006) has shed light on the factors involved in host-parasite communication in AE. *E. multilocularis* metacystodes express a number of developmental

factors which they share with other metazoans. These include signalling systems which employ receptor tyrosine kinases of the epidermal growth factor (EGF, Spiliotis *et al.* 2003, 2006), the insulin/insulin-like growth factor (Ins/IGF)-receptor families (Konrad *et al.* 2003) and the surface serine/threonine kinases of the closely-related transforming growth factor-beta (TGF- β) and bone morphogenetic protein (BMP)-receptor families (Zavalo-Gongora *et al.* 2006). A cytokine of *E. multilocularis* which has significant homology to mammalian EGF is substantially upregulated in metacestodes cultured under conditions that promote growth and differentiation (Spiliotis *et al.* 2003). Also, EmSkip, a novel member of the SNW/SKIP family of transcriptional co-regulators was shown to be expressed in the *Echinococcus* metacestodes (including protoscoleces) during an infection of the intermediate host (Gelmedin *et al.* 2005). EmSkip interacts with EmSmadA and EmSmadB, two TGF- β /BMP signal transducers of *E. multilocularis* (see Zavalo-Gongora *et al.* 2003), indicating a role for this protein in TGF- β signalling processes in this parasite. In addition, the downstream signalling elements of the MAP kinase cascade have been identified and characterized (Spiliotis and Brehm, 2004; Spiliotis *et al.* 2005, 2006). The *Echinococcus* MAP kinase cascade factors share molecular similarities to, but also differ in particular aspects from, their mammalian counterparts, and thus represent prime candidate targets for the development of novel anthelmintic drugs. This has been further substantiated *via* analysis of receptor activation, which has shown that the *E. multilocularis* insulin receptor EmIR interacts readily with insulin from the host, and TGF- β receptor EmTR1 and possibly also the EGF-receptor EmER can interact with their corresponding host ligands (reviewed by Brehm *et al.* 2006). Thus, parasite and host have evolved means of communication that would largely influence the developmental biology of both parasite and host. The evidence indicates that these receptor-ligand systems play a central role in host-parasite interaction processes and thus represent interesting drug targets (Brehm *et al.* 2006). Cancer research has generated an enormous number of compounds which interfere with the functional activities of homologous receptors or respective downstream kinases (see Sioud and Leirdal, 2007), and it will be the challenge now to identify drugs, or derivatives, which inhibit these receptors or the corresponding downstream enzymes in a parasite-specific manner.

(iv) *In silico* approaches

Mathis *et al.* (2005) employed current genomic sequence information to define a drug target in *Echinococcus in silico*, and subsequently confirmed

their hypothesis experimentally. In bacteria, the ribosomes are important antibiotic targets, and macrolides such as erythromycin and clarithromycin are agents which bind to the nascent peptide exit tunnel near the peptidyltransferase centre of large subunit ribosomal RNA (rRNA) (Rodriguez-Fonseca *et al.* 1995). Higher eukaryotes carry a guanine at position 2058 of both cytoplasmic and mitochondrial rRNAs, and this modification at this position has been demonstrated to confer the resistance of eukaryotic cells to macrolide antibiotics. In contrast, the mitochondrial rRNA of *E. multilocularis* carries an adenine at sequence position 2058, which would be predictive for susceptibility (Sander *et al.* 1997), while the nucleus-encoded rRNA is characterized by a guanine at 2058 (Mathis *et al.* 2005). Upon *in vitro* culture of *E. multilocularis* metacestodes with clarithromycin, parasites, as expected, exhibited severely impaired growth characteristics, presented morphologically altered mitochondria and displayed a lack of microtriches, all in a dose-dependent manner. Adult worms were also severely affected, lost their motility and displayed morphological alterations, such as shortening and constriction of proglottids and increased vacuolization. Mathis *et al.* (2005) were the first workers to employ a sequence-based *in silico* approach for the exploration of drugs whose mode of action was well studied at the molecular level and whose corresponding target was precisely defined. However, a prerequisite for such an approach is the availability of comprehensive genome sequence information for *Echinococcus* species.

CONCLUDING REMARKS

As outlined in this review, considerable efforts have been made to improve the therapeutical options for the treatment of CE and AE. The benzimidazole-based treatment regimens have improved considerably the prognosis of patients but, due to the obvious setbacks, new developments are needed. Compounds that not only act as parastatics but also as parasitocidals *in vivo* have not been discovered to date, but it is conceivable that such compounds actually exist. Thus, the current situation for affected patients is far from satisfactory. Academic institutions can provide a scientific basis that could eventually lead to novel treatment options, but financial constraints constantly limit the further development of promising avenues. Thus, considerably more input and support is needed from the pharmaceutical and biotechnological industries as well as governmental agencies to provide solutions for these neglected diseases. The novel genomic resources developing the discovery of the receptor-ligand interactions and associated signalling pathways which influence the parasite-host interaction, and the further characterization of immuno-modulatory molecules provide

new and exciting opportunities and promising targets for future studies of novel chemotherapeutical and immuno-therapeutical options for CE and AE.

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REFERENCES

- Ali-Khan, Z., Siboo, R., Gomersall, M. and Faucher, M.** (1983). Cystolytic events and the possible role of germinal cells in metastasis in chronic alveolar hydatidosis. *Annals of Tropical Medicine and Parasitology* **77**, 497–512.
- Ammann, R. and Eckert, J.** (1995). Clinical diagnosis and treatment of echinococcosis in humans. In *Echinococcosis and Hydatid Disease* (ed. Thompson, R. C. A. and Lymbery, A. J.), pp. 411–430. CAB International, Wallingford, UK.
- Bartholomot, G., Vuitton, D. A., Harraga, S., Shi, D. Z., Giraudoux, P., Barnish, G., Wang, Y. H., MacPherson, C. N. and Craig, P. S.** (2002). Combined ultrasound and serologic screening for hepatic alveolar echinococcosis in central China. *American Journal for Tropical Medicine and Hygiene* **66**, 23–29.
- Baz, A., Ettlin, G. M. and Dematteis, S.** (2006). Complexity and function of cytokine responses in experimental infection by *Echinococcus granulosus*. *Immunobiology* **211**, 3–9.
- Blanton, R. E., Wachira, T. M., Zeyhle, E., Njoroge, E. M., Magambo, J. K. and Schantz, P. M.** (1998). Oxfendazole treatment of cystic hydatid disease in naturally infected animals. *Antimicrobial Agents and Chemotherapy* **42**, 601–605.
- Brehm, K., Kronthaler, K., Jura, H. and Frosch, M.** (2000). Cloning and characterization of beta-tubulin genes from *Echinococcus multilocularis*. *Molecular and Biochemical Parasitology* **107**, 297–302.
- Brehm, K., Spiliotis, M., Zavala-Gongora, R., Konrad, C. and Frosch, M.** (2006). The molecular mechanisms of larval cestode development: first steps into an unknown world. *Parasitology International* **55**, S15–S21.
- Bresson-Hadni, S., Laplante, J. J., Lenys, D., Rohmer, P., Gottstein, B., Jacquier, P., Mercet, P., Meyer, J. P., Miguet, J. P. and Vuitton, D. A.** (1994). Sero-epidemiologic screening of *Echinococcus multilocularis* infection in a European area endemic for alveolar echinococcosis. *American Journal of Tropical Medicine and Hygiene* **51**, 837–846.
- Brunetti, E., Troia, G., Garlaschelli, A. L., Gulizia, R. and Filice, C.** (2004). Twenty years of percutaneous treatments for cystic echinococcosis: a preliminary assessment of their use and safety. *Parassitologia* **46**, 367–370.
- Casado, N., Urrea-Paris, M. A., Moreno, M. J., and Rodriguez-Caabeiro, F.** (2001). Combined praziquantel and albendazole chemoprophylaxis in experimental hydatidosis. *Parasitology Research* **87**, 787–789.
- Chai, J., Menghebat, Wie, J., Deyu, S., Bin, L., Jincao, S., Chen, F., Xiong, L., Yiding, M., Xiuling, W., Dolikun, Guliber, Yanchun, W., Fanghua, G. and Shuhua, X.** (2004). Observations on clinical efficacy of albendazole emulsion in 264 cases of hepatic cystic echinococcosis. *Parasitology International* **53**, 3–10.
- Cobo, F., Yarnoz, C., Sesma, B., Fraile, P., Aizcorbe, M., Trujillo, R., Diaz-de-Liano, A. and Ciga, M. A.** (1998). Albendazole plus praziquantel versus albendazole alone as preoperative treatment in intra-abdominal hydatidosis caused by *Echinococcus granulosus*. *Tropical Medicine and International Health* **3**, 462–466.
- Craig, P.** (2003). *Echinococcus multilocularis*. *Current Opinion in Infectious Diseases* **16**, 437–444.
- Dai, W. J., Hemphill, A., Waldvogel, A., Ingold, K., Deplazes, P., Mossmann, H. and Gottstein, B.** (2001). Major carbohydrate antigen of *Echinococcus multilocularis* induces an immunoglobulin G response independent of alpha beta(+) CD4(+) T cells. *Infection and Immunity* **69**, 6074–6083.
- Dai, W. J., Waldvogel, A., Siles-Lucas, M. and Gottstein, B.** (2004). *Echinococcus multilocularis* proliferation in mice and respective parasite 14-3-3 gene expression is mainly controlled by an alphabeta CD4 T-cell-mediated immune response. *Immunology* **112**, 481–488.
- Dakora, F. D. and Phillips, D. A.** (1996). Diverse functions of isoflavonoids in legumes transcend anti-microbial definitions of phytoalexins. *Physiological and Molecular Plant Pathology* **49**, 1–20.
- Dematteis, S., Piroto, F., Marques, J., Nieto, A., Orn, A. and Baz, A.** (2001). Modulation of the cellular immune response by a carbohydrate rich fraction from *Echinococcus granulosus* protoscoleces in infected or immunized Balb/c mice. *Parasite Immunology* **23**, 1–9.
- Dematteis, S., Rottenberg, M. and Baz, A.** (2003). Cytokine response and outcome of infection depends on the infective dose of parasites in experimental infection by *Echinococcus granulosus*. *Parasite Immunology* **25**, 189–197.
- Diaz, A., Irigoien, R., Ferreira, F. and Sim, R. B.** (1999). Control of host complement activation by the *Echinococcus granulosus* hydatid cyst. *Immunopharmacology* **42**, 91–98.
- Dueger, E. L., Moro, P. L. and Gilman, R. H.** (1999). Oxfendazole treatment of sheep with naturally acquired hydatid disease. *Antimicrobial Agents and Chemotherapy* **43**, 2263–2267.
- Eckert, J. and Deplazes, P.** (2004). Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clinical Microbiology Reviews* **17**, 107–135.
- Eckert, J., Thompson, R. C. A. and Mehlhorn, H.** (1983). Proliferation and metastases formation of larval *Echinococcus multilocularis*. I. Animal model, macroscopical and histological findings. *Zeitschrift für Parasitenkunde* **69**, 737–748.

- El-On, J.** (2002). Benzimidazole treatment of cystic echinococcosis. *Acta Tropica* **85**, 243–252.
- Emery, I., Leclerc, C., Sengphommachanh, K., Vuitton, D. A. and Liance, M.** (1998). *In vivo* treatment with recombinant IL-12 protects C57BL/6 mice against secondary alveolar echinococcosis. *Parasite Immunology* **20**, 81–91.
- Esposito, M., Mueller, N. and Hemphill, A.** (2007). Structure-activity relationships from *in vitro* efficacies of the thiazolide series against the intracellular apicomplexan protozoan *Neospora caninum*. *International Journal for Parasitology* **37**, 183–190.
- Ferreira, A. M., Irigoin, F., Breijo, M., Sim, R. B. and Diaz, A.** (2000). How *Echinococcus granulosus* deals with complement. *Parasitology Today* **16**, 168–172.
- Ferreira, A. M., Diaz, A., Fernandez, C. and Sim, R. B.** (2001). Assessment of *in vivo* complement activation on the *Echinococcus granulosus* hydatid cyst wall. *Parasite Immunology* **23**, 655–658.
- Fraize, M., Sarciron, M. E., Azzouz, S., Issadi, N., Bosquet, G. and Petavy, A. F.** (2005). Immunogenicity of two *Echinococcus granulosus* antigens EgA31 and EgTrp in mice. *Parasitology Research* **96**, 113–120.
- Frayha, G. J., Bikhazi, K. J. and Kachachi, T. A.** (1981). Treatment of hydatid cysts (*Echinococcus granulosus*) by Cetrimide. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 447–450.
- Garcia-Llamazares, J. L., Alvarez-de-Felipe, A. I., Redondo-Cardena, P., Voces-Alonso, J. A. and Prieto-Fernandez, J. G.** (1997). *In vivo* inhibition of the regenerative capacity of hydatid material after treatment with netobimin. *Parasitology Research* **83**, 105–108.
- Gelmedin, V., Zavala-Gongora, R., Fernandez, C. and Brehm, K.** (2005). *Echinococcus multilocularis*: cloning and characterization of a member of the SNW/SKIP family of transcriptional coregulators. *Experimental Parasitology* **111**, 115–120.
- Godot, V., Harraga, S., Podoprigora, G., Liance, M., Bardonnet, K. and Vuitton, D. A.** (2003). IFN- α -2a protects mice against helminth infection of the liver and modulates immune responses. *Gastroenterology* **124**, 1441–1450.
- Gottstein, B., Dai, W. J., Walker, M., Stettler, M., Muller, N. and Hemphill, A.** (2002). An intact laminated layer is important for the establishment of secondary *Echinococcus multilocularis* infection. *Parasitology Research* **88**, 822–828.
- Gottstein, B., Haag, K., Walker, M., Matsumoto, J., Mejri, N. and Hemphill, A.** (2006). Molecular survival strategies of *Echinococcus multilocularis* in the murine host. *Parasitology International* **55**, S45–S49.
- Gottstein, B. and Hemphill, A.** (1997). Immunopathology of echinococcosis. *Chemical Immunology* **66**, 177–208.
- Grenard, P., Bresson-Hadni, S., El Alaoui, S., Chevallier, M., Vuitton, D. A. and Ricard-Blum, S.** (2001). Transglutaminase-mediated crosslinking is involved in the stabilization of extracellular matrix in human liver fibrosis. *Journal of Hepatology* **35**, 367–375.
- Harraga, S., Godot, V., Bresson-Hadni, S., Pater, C., Beurton, I., Bartholomot, B. and Vuitton, D. A.** (1999). Clinical efficacy of and switch from T helper 2 to T helper 1 cytokine profile after interferon α 2a monotherapy for human echinococcosis. *Clinical Infectious Diseases* **29**, 205–206.
- Hemphill, A., Mueller, J. and Esposito, M.** (2006). Nitazoxanide, a broad-spectrum thiazolide anti-infective agent for the treatment of gastrointestinal infections. *Expert Opinion in Pharmacotherapy* **7**, 953–964.
- Hemphill, A., Stettler, M., Walker, M., Siles-Lucas, M., Fink, R. and Gottstein, B.** (2002). Culture of *Echinococcus multilocularis* metacystodes; an alternative to animal use. *Trends in Parasitology* **18**, 445–449.
- Horton, R. J.** (1989). Chemotherapy of *Echinococcus* infection in man with albendazole. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83**, 97–102.
- Horton, R. J.** (1997). Albendazole in the treatment of human cystic echinococcosis: 12 years of experience. *Acta Tropica* **64**, 79–93.
- Hurd, H., Mackenzie, K. S. and Chappell, L. H.** (1993). Anthelmintic effects of cyclosporin A on protoscoleces and secondary hydatid cysts of *Echinococcus granulosus* in the mouse. *International Journal for Parasitology* **23**, 315–320.
- Jenne, L., Kilwinski, J., Radloff, P., Flick, W. and Kern, P.** (1998). Clinical efficacy of immunologic alterations caused by interferon gamma therapy for alveolar echinococcosis. *Clinical Infectious Diseases* **26**, 492–494.
- Kern, P.** (2003). *Echinococcus granulosus* infection: clinical presentation, medical treatment and outcome. *Langenbecks Archives of Surgery* **388**, 413–420.
- Kern, P.** (2006). Medical treatment of echinococcosis under the guidance of Good Clinical Practice (GCP/ICH). *Parasitology International* **55** (Suppl.), S273–S282.
- Klauber, N., Parangi, S., Flynn, E., Hamel, E. and D'Amato, R. J.** (1997). Inhibition of angiogenesis and breast cancer in mice by the microtubule inhibitors 2-methoxyestradiol and taxol. *Cancer Research* **57**, 81–86.
- Klinkert, M. Q. and Heussler, V.** (2006). The use of anticancer drugs in antiparasitic chemotherapy. *Mini Reviews in Medicinal Chemistry* **6**, 131–143.
- Konrad, C., Kroner, A., Spiliotis, M., Zavala-Gongora, R. and Brehm, K.** (2003). Identification and molecular characterisation of a gene encoding a member of the insulin receptor family in *Echinococcus multilocularis*. *International Journal for Parasitology* **33**, 301–312.
- Kumar, A. P., Garcia, G. E., Orsborn, J., Levin, V. A., and Slaga, T. J.** (2003). 2-Methoxyestradiol interferes with Nf κ B transcriptional activity in primitive neuroectodermal brain tumours: implications for management. *Carcinogenesis* **24**, 209–216.
- Launchbury, A. P. and Habboubi, N.** (1993). Epirubicin and doxorubicin: a comparison of their characteristics, therapeutic activity and toxicity. *Cancer Treatment Reviews* **19**, 197–228.
- Lawton, P., Walchshofer, N. and Sarciron, M. E.** (2001). *In vitro* effects of isoprinosine and a dipeptide methyl ester on *Echinococcus multilocularis* protoscoleces. *Journal of Helminthology* **75**, 251–257.

- Liance, M., Bresson-Hadni, S., Vuitton, D. A., Lenys, D., Carbillet, J. P. and Houin, R.** (1992). Effects of cyclosporin A on the course of murine alveolar echinococcosis and on specific cellular and humoral immune responses against *Echinococcus multilocularis*. *International Journal for Parasitology* **22**, 23–28.
- Liance, M., Nemati, F., Bories, C. and Couvreur, P.** (1993). Experience with doxorubicin-bound polyisohexylcyanoacrylate nanoparticles on murine alveolar echinococcosis of the liver. *International Journal for Parasitology* **23**, 427–429.
- Liance, M., Ricard-Blum, S., Emery, I., Houin, R. and Vuitton, D. A.** (1998). *Echinococcus multilocularis* infection in mice: *in vivo* treatment with a low dose of IFN-gamma decreases metacestode growth and liver fibrogenesis. *Parasite* **5**, 231–237.
- Mabjeesh, N. J., Escuin, D., LaVallee, T. M., Pribluda, V. S., Swartz, G. M., Johnson, M. S., Willard, M. T., Zhong, H., Simons, J. W. and Giannakakou, P.** (2003). 2-ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. *Cancer Cell* **3**, 363–375.
- MacPherson, C. N., Kachani, M., Lyagoubi, M., Berrada, M., Shepherd, M., Fields, P. F. and El Hasnaoui, M.** (2004). Cystic echinococcosis in the Berber of the Mid Atlas mountains, Morocco: new insights into the natural history of the disease in humans. *Annals of Tropical Medicine and Parasitology* **98**, 481–490.
- Maizels, R. M. and Yazdanbakhsh, M.** (2003). Immune regulation by helminth parasites: Cellular and molecular mechanisms. *Nature Reviews Immunology* **3**, 733–744.
- Manfras, B., Reuter, S., Wendland, T. and Kern, P.** (2002). Increased activation and oligoclonality of peripheral CD8⁺ T cells in the chronic human helminth infection alveolar echinococcosis. *Infection and Immunity* **70**, 1168–1174.
- Martinez, J., Perez-Serrano, J., Bernadina, W. E. and Rodriguez-Caabeiro, F.** (1999). *Echinococcus granulosus*: *in vivo* effects of ivermectin and praziquantel on hsp60 and hsp70 levels. *Experimental Parasitology* **93**, 171–180.
- Marchiondo, A. A., Ming, R., Andersen, F. L., Slusser, J. H. and Conder, G. A.** (1994). Enhanced larval cyst growth of *Echinococcus multilocularis* in praziquantel-treated jirds (*Meriones unguiculatus*). *American Journal of Tropical Medicine and Hygiene* **50**, 120–127.
- Mathis, A., Wild, P., Boettger, E. C., Kapel, C. M. O. and Deplazes, P.** (2005). Mitochondrial ribosome as the target for the macrolide antibiotic clarithromycin in the helminth *Echinococcus multilocularis*. *Antimicrobial Agents and Chemotherapy* **49**, 3251–3255.
- McManus, D. P., Wenbao, Z., Jun, L. and Bartley, P. B.** (2003). Echinococcosis. *The Lancet* **362**, 1295–1304.
- Mehlhorn, H., Eckert, J., and Thompson, R. C. A.** (1983). Proliferation and metastases formation of larval *Echinococcus multilocularis* II. Ultrastructural investigations. *Zeitschrift für Parasitenkunde* **69**, 749–763.
- Messina, M.** Soy, soy phytoestrogens (isoflavones), and breast cancer. *American Journal of Clinical Nutrition* **70**, 574–575.
- Miyaji, S., Katakura, K., Matsufuji, S., Murakami, Y., Hayashi, S., Oku, Y., Okamoto, M. and Kamiya, M.** (1993). Failure of treatment with alpha-difluoromethylornithine against secondary multilocular echinococcosis in mice. *Parasitology Research* **79**, 75–76.
- Moro, P. L., Garcia, H. H., Gonzales, A. E., Bonilla, J. J., Verastegui, M. and Gilman, R. H.** (2005). Screening for cystic echinococcosis in an endemic region of Peru using portable ultrasonography and the enzyme linked immunoelectrotransfer blot (EITB) assay. *Parasitology Research* **96**, 242–246.
- Morris, D. L., and Taylor, D. H.** (1990). *Echinococcus granulosus*: development of resistance to albendazole in an animal model. *Journal of Helminthology* **64**, 171–174.
- Naguleswaran, A., Spicher, M., Vonlaufen, N., Ortega-Mora, L. M., Torgerson, P., Gottstein, B. and Hemphill, A.** (2006). *In vitro* metacestodicidal activities of genistein and other isoflavones against *Echinococcus multilocularis* and *Echinococcus granulosus*. *Antimicrobial Agents and Chemotherapy* **50**, 3770–3778.
- Ohbayashi, M., Rausch, R. L. and Fay, F. H.** (1971). On the ecology and distribution of *Echinococcus* spp. (Cestoda: Taeniidae), and characteristics of their development in the intermediate host. II. Comparative studies on the larval *E. multilocularis* Leuckart, 1863, in the intermediate host. *Japanese Journal of Veterinary Research*, **19**, 1–9.
- Ortona, E., Margutti, P., Delunardo, F., Vaccari, S., Rigano, R., Profumo, E., Buttari, B., Teggi, A. and Siracusano, A.** (2003). Molecular and immunological characterization of the C-terminal region of a new *Echinococcus granulosus* heat shock protein 70. *Parasite Immunology* **25**, 119–126.
- Pawloski, Z. S.** (1997). Critical points in the clinical management of cystid echinococcosis: a revised review. In *Compendium on Cystic Echinococcosis* (ed. Anderson, F. L., Ouhelli, H. and Kachani, M.), pp. 119–135. Brigham Young University, Provo, USA.
- Pawloski, Z. S., Eckert, J., Vuitton, D. A., Ammann, R. W., Kern, P., Craig, P. S., Dar, K. F., De Rosa, F., Filice, C., Gottstein, B., Grimm, F., MacPhearson, C. N. L., Sato, N., Todorov, T., Uchino, J., von Sinner, W. and Wen, H.** (2001). Echinococcosis in humans: clinical aspects, diagnosis and treatment. In *WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern* (ed. Eckert, J., Gemmel, M. A., Meslin, F. X. and Pawlowski, Z. S), pp. 20–32. World Organization for Animal Health and World Health Organization, Paris, France.
- Rau, M. E. and Tanner, C. E.** (1975). BCG suppresses growth and metastasis of hydatid infections. *Nature, London* **256**, 318–319.
- Rausch, R. L., Wilson, J. F., Schantz, P. M. and McMahan, B. J.** (1987). Spontaneous death of *Echinococcus multilocularis*: case diagnosed serologically (by Em2-Elisa) and clinical significance. *American Journal of Tropical Medicine and Hygiene* **36**, 576–585.

- Rausch, R. L.** (1995). Life cycle patterns and geographic distribution of *Echinococcus* species. In *Echinococcosis and Hydatid Disease* (ed. Thompson, R. C. A. and Lymbery, A. J.), pp. 89–99. CAB International, Wallingford, UK.
- Reuter, S., Buck, A., Grebe, O., Nüssle-Kügele, K., Kern, P. and Manfras, B.** (2003b). Salvage treatment with amphotericin B in progressive human alveolar echinococcosis. *Antimicrobial Agents and Chemotherapy* **47**, 3586–3591.
- Reuter, S., Buck, A., Manfras, B., Kratzer, W., Seitz, H. M., Darge, K., Reske, S. N. and Kern, P.** (2004). Structured treatment interruption in patients with alveolar echinococcosis. *Hepatology* **39**, 509–517.
- Reuter, S., Jensen, B., Buttenschoen, K., Kratzer, W. and Kern, P.** (2000). Benzimidazoles in the treatment of alveolar echinococcosis. *Journal of Antimicrobial Chemotherapy* **46**, 451–460.
- Reuter, S., Manfras, B., Merkle, M., Harter, G. and Kern, P.** (2006). *In vitro* activities of itraconazole, methiazole, and nitazoxanide versus *Echinococcus multilocularis* larvae. *Antimicrobial Agents and Chemotherapy* **50**, 2966–2970.
- Reuter, S., Merkle, M., Brehm, K., Kern, P. and Manfras, B.** (2003a). Effect of amphotericin B on larval growth of *Echinococcus multilocularis*. *Antimicrobial Agents and Chemotherapy* **47**, 620–625.
- Reynaud, J., Guilet, D., Terreux, R., Lussignol, M. and Walchshofer, N.** (2005). Isoflavonoids in non-leguminous families: an update. *Natural Products Reports* **22**, 504–515.
- Rigano, R., Profumo, E., Ioppolo, S., Notargiacomo, S., Ortona, E., Teggi, A. and Siracusano, A.** (1995). Immunological markers indicating the effectiveness of pharmacological treatment in human hydatid disease. *Clinical Experimental Immunology* **102**, 281–285.
- Rigano, R., Buttari, B., De Falco, E., Profumo, E., Ortana, E., Margutti, P., Scotta, C., Teggi, A. and Siracusano, A.** (2004). *Echinococcus granulosus*-specific T cell lines derived from patients at various clinical stages of cystic echinococcosis. *Parasite Immunology* **26**, 45–52.
- Rodrigues, J. M., Bories, C., Emery, I., Fessi, H., Devissaguet, J. P. and Liance, M.** (1995). Development of an injectable formulation of albendazole and *in vivo* evaluation of its efficacy against *Echinococcus* metacestodes. *International Journal for Parasitology* **25**, 1437.
- Rodriguez-Fonseca, C., Amils, R. and Garret, R. A.** (1995). Fine structure of the peptidyl transferase centre on 23S-like rRNAs deduced from chemical probing of antibiotic ribosome complexes. *Journal of Molecular Biology* **27**, 224–235.
- Rogan, M. T. and Richards, K. S.** (1986). *Echinococcus granulosus*: *in vivo* effect of monensin on the tegument of the protoscolex. *Parasitology* **93**, 347–355.
- Sander, P. T., Prammananan, T., Meier, A., Frischkorn, K. and Bottger, E. C.** (1997). The role of ribosomal RNAs in macrolide resistance. *Molecular Microbiology* **26**, 469–480.
- Sarciron, M. E., Delabre, I., Walbaum, S., Raynaud, G., and Petavy, A. F.** (1992). Effects of multiple doses of isoprinosine on *Echinococcus multilocularis* metacestodes. *Antimicrobial Agents and Chemotherapy* **36**, 191–194.
- Sarciron, M. E., Walbaum, S., Arzac, C., Raynaud, G., and Petavy, A. F.** (1993). *Echinococcus granulosus*: isoprinosine treatment of the metacestode stage. *American Journal of Tropical Medicine and Hygiene* **48**, 658–665.
- Sarciron, M. E., Walbaum, M. E. and Petavy, A. F.** (1995). Effects of Isoprinosine on *Echinococcus multilocularis* and *E. granulosus* metacestodes. *Parasitology Research* **81**, 329–333.
- Sarciron, M. E., Walchshofer, N., Walbaum, S., Arzac, C., Descotes, J., Petavy, A. F. and Paris, J.** (1997). Increases in the effects of albendazole on *Echinococcus multilocularis* metacestodes by the dipeptide methyl ester (Phe-Phe-OMe). *American Journal of Tropical Medicine and Hygiene* **56**, 226–230.
- Schantz, P. M., Chai, J., Craig, P. S., Eckert, J., Jenkins, D. J., Macpherson, C. N. L. and Thakur, A.** (1995). Epidemiology and control of hydatid disease. In *Echinococcosis and Hydatid Disease* (ed. Thompson, R. C. A. and Lymbery, A. J.), pp. 233–245. CAB International, Wallingford, UK.
- Schmid, M., Samonigg, H., Stoger, H., Auer, H., Sternthal, M. H., Wilders-Truschnig, M. and Reisinger, E. C.** (1995). Use of interferon-gamma and mebendazole to stop the progression of alveolar hydatid disease: case report. *Clinical Infectious Diseases* **20**, 1543–1546.
- Schumacher, G. and Neuhaus, P.** (2001). The physiological estrogen metabolite 2-methoxyestradiol reduces tumor growth and induces apoptosis in human solid tumors. *Journal of Cancer Research and Clinical Oncology* **127**, 405–410.
- Siles-Lucas, M., Felleisen, R. S., Hemphill, A., Wilson, W. and Gottstein, B.** (1998). Stage-specific expression of the 14-3-3 gene in *Echinococcus multilocularis*. *Molecular and Biochemical Parasitology* **91**, 281–293.
- Siles-Lucas, M. and Gottstein, B.** (2003). The 14-3-3 protein: a key molecule in parasites as in other organisms. *Trends in Parasitology* **19**, 575–581.
- Siles-Lucas, M. and Hemphill, A.** (2002). Cestode parasites: Application of *in vivo* and *in vitro* models for studies on the host-parasite relationship. *Advances in Parasitology* **51**, 133–230.
- Siles-Lucas, M., Nunes, C. P. and Zaha, A.** (2001). Comparative analysis of the 14-3-3 gene and its expression in *Echinococcus granulosus* and *Echinococcus multilocularis* metacestodes. *Parasitology* **122**, 281–287.
- Sioud, M. and Leirdal, M.** (2007). Druggable signaling proteins. *Methods in Molecular Biology* **361**, 1–24.
- Spiliotis, M. and Brehm, K.** (2004). *Echinococcus multilocularis*: identification and molecular characterization of a Ral-like small GTP-binding protein. *Experimental Parasitology* **107**, 163–172.
- Spiliotis, M., Konrad, C., Gelmedin, V., Tappe, D., Bruckner, S., Mosch, H. U. and Brehm, K.** (2006). Characterisation of EmMPK1, an ERK-like MAP kinase from *Echinococcus multilocularis* which is activated in response to human epidermal growth factor. *International Journal for Parasitology* **36**, 1097–1112.
- Spiliotis, M., Kroner, A. and Brehm, K.** (2003). Identification, molecular characterization and

- expression of the gene encoding the epidermal growth factor receptor orthologue from the fox-tapeworm *Echinococcus multilocularis*. *Gene* **323**, 57–65.
- Spiliotis, M., Tappe, D., Bruckner, S., Mosch, H. U. and Brehm, K.** (2005). Molecular cloning and characterization of Ras- and Raf-homologues from the fox-tapeworm *Echinococcus multilocularis*. *Molecular and Biochemical Parasitology* **139**, 225–237.
- Spiliotis, M., Tappe, D., Sesterhenn, L. and Brehm, K.** (2004). Long-term *in vitro* cultivation of *Echinococcus multilocularis* metacestodes under axenic conditions. *Parasitology Research* **92**, 430–432.
- Stettler, M., Fink, R., Walker, M., Gottstein, B., Geary, T. G., Rossignol, J. F. and Hemphill, A.** (2003). *In vivo* parasitocidal effect of nitazoxanide against *Echinococcus multilocularis* metacestodes. *Antimicrobial Agents and Chemotherapy* **47**, 467–474.
- Stettler, M., Rossignol, J. F., Fink, R., Walker, M., Gottstein, B., Merli, M., Theurillat, R., Thormann, W., Dricot, E., Segers, R. and Hemphill, A.** (2004). Secondary and primary murine alveolar echinococcosis: combined albendazole/nitazoxanide chemotherapy exhibits profound anti-parasitic activity. *International Journal for Parasitology* **34**, 615–624.
- Stey, C. and Jost, R.** (1993). Rezidivierende, lebensbedrohliche Anaphylaxie als Erstmanifestation eines *Echinococcus cysticus (granulosus)* der Leber. *Schweizerische Medizinische Wochenschrift*, **123**, 1445–1450.
- Taylor, D. H., Richards, K. S. and Morris, D. L.** (1989). Rapid recovery of *Echinococcus granulosus* following 'successful' albendazole therapy in a gerbil model. *Journal of Helminthology* **63**, 349–352.
- Thompson, R. C. A.** (1986). Biology and systematics of *Echinococcus*. In *The Biology of Echinococcus and Hydatid Disease* (ed. Thompson, R. C. A.), pp. 5–17. Allen and Unwin, London.
- Torgerson, P. R.** (2003). Economic effects of echinococcosis. *Acta Tropica* **85**, 113–118.
- Urrea-Paris, M. A., Casado, N., Moreno, M. J. and Rodriguez-Caabeiro, F.** (2001). Chemoprophylactic praziquantel treatment in experimental hydatidosis. *Parasitology Research* **87**, 510–512.
- Urrea-Paris, M. A., Moreno, M. J., Casado, N. and Rodriguez-Caabeiro, F.** (1999). *Echinococcus granulosus*: praziquantel treatment against the metacestode stage. *Parasitology Research* **85**, 999–1006.
- Vuitton, D. A., Bresson-Hadni, S., Laroche, L., Kaiserlian, D., Guerret-Stocker, S., Bresson, J. L. and Gilbert, M.** (1989). Cellular immune response in *Echinococcus multilocularis* infection in humans. II. Natural killer cell activity and cell subpopulations in the blood and in the periparasitic granuloma of patients with alveolar echinococcosis. *Clinical and Experimental Immunology* **78**, 67–74.
- Vuitton, D. A., Zhang, S. L., Yang, Y., Godot, V., Beurton, I., Manton, G., Bresson-Hadni, S.** (2006). Survival strategy of *Echinococcus multilocularis* in the human host. *Parasite International* **55**, S51–S55.
- Walchshofer, N., Delabre-Defayolle, I., Paris, J. and Petavy, A. F.** (1990). *In vivo* morphological damage induced by a new benzimidazole pro-drug in *Echinococcus multilocularis* metacestodes. *Journal of Pharmacological Science* **79**, 606–608.
- Walker, M., Baz, A., Dematteis, S., Stettler, M., Gottstein, B., Schaller, J. and Hemphill, A.** (2004a). Isolation and characterization of a secretory component of *Echinococcus multilocularis* metacestodes potentially involved in modulating the host-parasite interface. *Infection and Immunity* **72**, 527–536.
- Walker, M., Rossignol, J. F., Torgerson, P. and Hemphill, A.** (2004b). *In vivo* effects of nitazoxanide on *Echinococcus granulosus* protoscoleces and metacestodes. *Journal of Antimicrobial Chemotherapy* **54**, 609–616.
- Wangoo, A., Ganguly, N. K. and Mahajan, R. C.** (1987). *In vivo* efficacy of mebendazole in containment of larval cyst mass in early stages of hydatid disease due to *Echinococcus granulosus*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 965–966.
- Wen, H., New, R. R., Muhmut, M., Wang, J. H., Wang, Y. H., Zhang, J. H., Shao, Y. M. and Craig, P. S.** (1996). Pharmacology and efficacy of liposome-entrapped albendazole in experimental secondary alveolar echinococcosis and effect of co-administration with cimetidine. *Parasitology* **113**, 111–121.
- Xiao, S., Feng, J. and Yao, M.** (1995). Effect of anti-hydatid drugs on carbohydrate metabolism of metacestode of *Echinococcus granulosus*. *Chinese Medical Journal* **108**, 682–688.
- Zavala-Gongora, R., Kroner, A., Bernthaler, P., Knaus, P. and Brehm, K.** (2006). A member of the transforming growth factor-beta receptor family from *Echinococcus multilocularis* is activated by human bone morphogenetic protein 2. *Molecular and Biochemical Parasitology* **146**, 265–271.
- Zavala-Gongora, R., Kroner, A., Wittek, B., Knaus, P. and Brehm, K.** (2003). Identification and characterisation of two distinct Smad proteins from the fox-tapeworm *Echinococcus multilocularis*. *International Journal for Parasitology* **33**, 1665–1677.
- Zhang, W. and McManus, D. P.** (2006). Recent advances in the immunology and diagnosis of echinococcosis. *FEMS Immunology and Medical Microbiology* **47**, 24–41.