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Hydatid Disease: Current Status of Chemotherapy and Drug Delivery Systems

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Abstract: Human echinococcosis or hydatid disease still causes serious health problems with a worldwide geographical distribution. This parasitic infection is a chronic, complex, and still neglected disease. Currently four treatment modalities are in use: surgery, PAIR (puncture, aspiration, injection of protoscolicidal agent, reaspiration), chemotherapy and a "watch and wait" approach for inactive, clinically silent cysts. Over the past 30 years, chemotherapy with benzimidazoles (BZ), like albendazole and mebendazole, has been used increasingly to treat this pathology. Unfortunately, 20%–40% of the cases do not respond favorably to such chemotherapy and these drugs produce stabilization, rather than cure in the majority of patients. However, the overall efficacy of BZ has been overstated in the past. With regard to these difficulties, novel therapeutical tools are needed to optimize treatment of human echinococcosis.

On the one hand, a number of compounds have been investigated, either using *in vitro* cultured parasites and/or applying to *in vivo* rodent models. Tested compounds include BZ derivatives such as flubendazole, and oxfendazole, as well as other anti-infective agents like ivermectin, nitazoxanide, genistein, artemisinin, timol, rapamycin, and anti-cancer agents such as 2-methoxyestradiol and cyclosporine A. Although some of these compounds showed promising activities *in vitro*, and also in the rodent models, they have not been yet translated to clinical applications.

On the other hand, different drug delivery systems have been developed in order to improve the efficacy of several active pharmaceutical ingredients (APIs) such as oil in water emulsion, liposomes and nanoparticles among others.

The present review article summarizes the chemotherapeutic state-of-the-art and the research done in the field of drug delivery systems regarded human echinococcosis.

Keywords: Chemotherapy, drug delivery, hydatid disease, therapy.

1. INTRODUCTION

Zoonoses, which are diseases and infections naturally transmitted between vertebrate animals and humans, account for most of the diseases that have been reported as emerging or re-emerging, as 75% of emerging human pathogens are zoonotic [1].

Helminths are the most common infectious agents of humans in developing countries [2]. Human echinococcosis or hydatid disease is a zoonotic infection caused by larval forms (metacestodes) of tapeworms of the genus Echinococcus found in the small intestine of carnivores. This disease is included by the WHO in the list of the neglected tropical diseases, in which the use of integrated approaches to cure, prevent and control the disease at the human-animal interface is needed in order to avoid its dissemination [3].

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Four distinct species within the genus *Echinococcus* have been identified: *Echinococcus granulosus, E. multilocularis, E. vogeli and E. oligarthrus*. All species are potentially zoonotic, but only two have significant medical and public health importance: *E. granulosus* and *E. multilocularis*, which cause cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively. The two other species, *E. vogeli* and *E. oligarthrus*, are responsible for polycystic echinococcosis in Central and South America. Few cases of this condition have been reported in man, and the real extent of the disease is unknown [4].

The global burden, as assessed by disability-adjusted life years (DALYs), for human CE was estimated to be 285,000 and an annual economic loss of US$ 194 million [5]. On the other hand, approximately 18,235 new cases of AE occur annually, with a total annual burden of 666,434 DALYs [6].

The purpose of this contribution is to review the chemotherapeutic state-of-the-art, the available new technologies and the research done in the field of drug delivery systems regarded CE and AE.

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2. CYSTIC ECHINOCOCCOSIS

2.1. Epidemiology

E. granulosus has a worldwide geographic distribution occurring in all continents including circumpolar, temperate, subtropical and tropical areas, and in at least 100 countries [7]. The highest prevalence is found in areas of Eurasia (specially Mediterranean countries, the Russian Federation and adjacent independent states, and China), north and east Africa, Australia, and South America. The actual prevalence of the parasite varies from sporadic to high, but only a few countries can be regarded as being free of *E. granulosus* [8]. *E. granulosus* comprises several different genotypes that may affect the epidemiology, pathology, and control of CE. To date, ten different genotypes (G1–G10) have been identified. Most *E. granulosus* isolates from human patients thus far have been of the sheep genotype (G1) [9]. The annual incidence of CE can range from less than 1 to 200 per 100,000 inhabitants in various endemic areas [10].

2.2. Life Cycle

The habitat of the adult worms is the small intestine of a carnivore (definitive host) such as dogs and wolves, where sexual reproduction and subsequent egg production take place. Eggs or gravid proglottids are shed in faeces. Following ingestion by a human or ungulate intermediate host (sheep, goats, pigs, cattle, horses, or camels) an oncosphere larva is released from the egg. The larvae then penetrate the intestinal mucosa and reach through the bloodstream various internal organs where the development of cysts occurs. Hydatid cysts develop mainly in the liver and lungs of intermediate hosts as unilocular fluid-filled bladders. Since the life cycle relies on carnivores eating infected herbivores, humans are usually a 'dead-end' for the parasite, although this is not always the case [11].

2.3. Course of Infection

Human disease in CE relates to the development and growth of fluid-filled cysts mainly in the liver and the lungs, although it can also affect the abdominal cavity, heart, bones, muscles, nervous system, or other locations [7].

About 5 days after eggs ingestion the metacestode is a small vesicle (60 to 70 mm in diameter) consisting on an internal cellular layer (germinal layer) and an outer acellular, laminated layer. Each cyst is surrounded by a host-produced layer of granulomatous adventitial reaction. Growth of cystic larvae is slow and well tolerated by the host, occasionally leading to large parasitic masses [12]. Most of the cysts are univesicular (i.e., unilocular), but in some of them, smaller daughter cysts are formed within larger mother cysts.

The initial phase of the primary infection is always asymptomatic. Small, well encapsulated, non progressive or calcified cysts typically do not induce major pathology, and patients may remain asymptomatic for years or permanently [13]. The induction of morbidity depends on the number, size, and developmental status of the cyst(s) (active or inactive), the involved organ, the localization of the cyst(s) within the organ, the pressure of cysts on surrounding tissues and structures, and the defense mechanisms of the infected individual. Clinical signs may occur after a highly variable incubation period, which can last several months or even years. Hepatic cysts can cause pain in the upper abdominal region, hepatomegaly, cholestasis, biliary cirrhosis, portal hypertension, ascites, and a variety of others manifestations [13]. Cysts may rupture into the peritoneal cavity, causing anaphylaxis or secondary CE, or into the biliary tree, leading to cholangitis and cholestasis. Abscess formation is possible after bacterial infection of cysts. Chronic cough, expectoration, dyspnea, hemoptysis, pleuritis, and lung abscess are selected symptoms caused by pulmonary cysts, and neurological disorders can be induced by cysts in the brain [7].

CE occurs in aged groups from younger than 1 to over 75 years old. In some areas of endemic infection, most hospital cases are recorded in the aged groups between 21 and 40 years old, but the highest morbidity may also occur in younger individuals aged between 6 and 20 years old [7].

2.4. Treatment

Depending on different factors such as cyst number and size and location, viability status, the involved organ and location, the interaction between the expanding parasite and the adjacent host tissue and bacterial and fungal infection, the options for treatment of CE are: surgery, percutaneous procedures, antiparasitic drug treatment or watch and wait. Furthermore, it must to be taken into account potential complications related to cyst rupture and spillage of protoscoleces, which can lead to an anaphylactic reaction or to a secondary CE [14, 15].The evidence supporting any of the four treatment modalities from carefully designed clinical studies, is insufficient, in consequence choosing treatment options for patients remains controversial [8].

2.4.1. Surgery

Surgery was the only option until 1980 and is still preferred in some cases, for example in those patients with limited cyst number and organ involvement, when cysts are not in risky locations, and the disease is not too far advanced. The procedure can be performed by open or laparoscopic surgery. The choice of surgical technique depends on the size, site, and type of the cyst(s), the existence of complications, and the surgeon´s experience [16]. Surgical excision is the preferred procedure because it represents the best treatment option to definitively cure the disease [17].

In CE, radical resection of the cyst mass represents the traditional treatment strategy and it is, in many instances, accompanied by chemotherapy. Protoscolicidal substances are often applied since there is a risk of spilling of cyst fluid containing protoscoleces, which would be responsible for metastasis formation or an anaphylactic reaction that could be fatal [14, 15, 18, 19]. In some cases, chemotherapeutic agents can be administered before surgery to reduce the size and number of viable protoscolices [20].

2.4.2. Percutaneous Treatments

Percutaneous techniques aim either to destroy the germinal layer with prostoscolicidal agents or to evacuate the entire endocyst. The most popular method in this group is PAIR. It is a minimally invasive technique used in the treatment of cysts in the liver and other abdominal locations. It is indicated for inoperable patients and those who refuse

surgery, in cases of relapse after surgery or failure to respond to BMZ alone. PAIR includes: (1) percutaneous puncture of cysts using ultrasonic guidance, (2) aspiration of cyst fluid, (3) injection of protoscolicidal substance (e.g. 95% ethanol) for 10–15 min and (4) re-aspiration of the fluid. ABZ should always be administered for at least 30 days after puncture. Modified catheterization techniques have been used for cysts, which are difficult to drain or tend to relapse after PAIR [8].

An alternative method to PAIR or surgery for the treatment of muscle-, spleen- or kidney-cysts include percutaneous drainage without reaspiration [21].

Although there is a risk of anaphylactic reaction associated to percutaneous drainage, specific complications are no more frequent than after surgery and fear of anaphylactic shock is no longer justified as an argument to avoid this therapeutic option [22]. Nevertheless, the efficacy and potential risks have not been fully evaluated, and more long-term studies are needed [23].

2.4.3. Chemotherapy

Medical treatment is indicated when surgical removal is not appropriate for patients with multiple cysts in two or more organs, for prevention of secondary echinococcosis after surgery and in some cases for presurgical treatment [13]. For inoperable cases, chemotherapy with benzimidazoles remains the most common option.

In general, several factors affect the response to medical treatment, such as size and age of the cysts, thickness of host derived connective tissue, calcification, cyst complication with multiple compartments or daughter cysts, ability of the drug to penetrate the cyst wall and persistence of adequate level of drug or its active metabolite at the site of parasite location (bone cysts are less sensitive) [7, 24, 25].

The benzimidazoles, albendazole (ABZ) and mebendazole (MBZ) are broad spectrum antiparasitic agents that have been widely used for several years in both humans and animals for echinococcosis treatment. Since the introduction of MBZ in mid 1970s, and ABZ in mid 1980s, there has been significant alteration in the medical and surgical management of human echinococcosis.

Different studies about CE treatment report that BZ administration and evaluation for up to 12 months, the cysts disappeared in 10–30% of the patients; 50–70% of the patients have demonstrated significant regression of the cyst size and alleviation of symptoms, and in 20–30% of patients *E. granulosus* metacestodes did not respond to chemotherapy [13, 26, 27].

Among this agents, the results for ABZ have been superior [13, 25] and is the first choice therapy in the treatment of CE and AE [28]. However, the curative effect of ABZ is not ideal because its strong hydrophobicity leads to poor absorption and low bioavailability.

As it was mentioned, anthelmintic therapy is not only administered when surgery is not possible, for example, preoperative albendazole is sometimes used to reduce the risk of dissemination during surgery [29].

MBZ and ABZ are generally well tolerated, but when they are used as long-term chemotherapy, they can produce several adverse reactions. Side effects usually include elevation of transaminases, proteinuria, loss of hair, gastrointestinal disturbances, neurological symptoms (vertigo/dizziness), leucopenia, headache, abnormal liver biopsy, abdominal pain, fever, urticaria, thrombocytopenia, and bone marrow toxicity [23]. Several studies have shown that MBZ and ABZ may induce embryotoxic or teratogenic effects, and so they are not at the early stages of pregnancy [26, 27, 30]. Constant monitoring of drug serum levels is suggested in order to avoid toxic reactions.

Praziquantel (PZQ), a synthetic isoquinolone-pyrazine derivative, is one of the first-line anthelmintic drugs for treating tapeworm infections and has become the cornerstone for hydatid control campaigns worldwide [31]. Although PZQ is a very effective anthelmintic, it is practically insoluble in water and has poor bioavailability, thus repeated administration of high doses for long period of time is required. For this reason, PZQ was proposed to be used alongside with BZ in CE-patients. The combined treatment with ABZ and PZQ given during the month prior to surgery increased the number of human patients with non-viable protoscoleces, as compared to therapy with ABZ alone [32]. The usefulness of PZQ in avoiding secondary echinococcosis needs further study [33].

2.4.4. Watch and Wait

In some cases the cysts do not produce any symptomatology and are incidentally detected. Some studies have suggested that inactive cysts that are free of complications can be safely monitored by ultrasound imaging without being treated. Prospective studies need to be performed to confirm the safety of this option [8].

3. ALVEOLAR ECHINOCOCCOSIS

3.1. Epidemiology

E. multilocularis has an extensive geographical distribution in the northern hemisphere, including endemic regions in central Europe, most of northern and central Eurasia (extending eastwards to Japan) and parts of North America [34]. The parasite has been reported in at least 30 countries, including Russia, Kazakhstan, and other areas of Central Asia, P.R. China, parts of Europe and North America [7]. The annual incidence of AE is generally low in most of the endemic areas (0.03–1.20 per 100,000 inhabitants) but in untreated or in inadequately treated patients mortality is more than 90% from 10 to 15 years of diagnosis [10].

3.2. Life Cycle

Adult worm infections of *E. multilocularis* are perpetuated in a sylvatic cycle with wild carnivores—mainly red (*Vulpes vulpes*) and arctic (*Alopex lagopis*) foxes—regarded as the most important definitive hosts. Domestic dogs and cats can also harbor the tapeworm and may be involved in a synanthropic cycle. Small mammals (usually microtine and arvicolid rodents) act as intermediate hosts. Larval growth in the liver remains indefinitely in the proliferative stage, resulting on the invasion of the surrounding tissues. It is thought that people become exposed to *E. multilocularis* by handling infected hosts, or by ingestion of food contaminated with eggs. Coprophagic

flies and other animals may serve as mechanical vectors of the eggs of both species [11].

3.3. Course of Infection

In contrast with CE, AE does not have well-defined external limits and infiltrates the surrounding parenchyma [10]. The larval mass proliferates rapidly by exogenous budding of germinative tissue and produces an alveolar-like pattern of microvesicles filled with protoscoleces. In humans, the larval mass resembles a malignancy in appearance and behaviour, because it proliferates indefinitely by exogenous budding and it invades the surrounding tissues. Protoscoleces are rarely observed in human infections [25].

3.4. Treatment

Radical surgery has been the historical cornerstone of treatment for AE. Early diagnosis of AE can result in a reduced rate of unresectable lesions and reduces the requirement for radical surgery. For the treatment of AE, BZ are always indicated after surgery when the resection is complete, and they should be administered for at least 2 years, and the patients should be monitored for 10 years to control recurrence [13]. On the other hand, chemotherapy is mandatory for inoperable AE and following incomplete surgical resection of the parasite lesions. In this case the treatment is long-term, often life-long, and it is based on ABZ and/or MBZ [35, 36]. Although BZ have theoretically the potential to cure patients with CE because they can kill *E. granulosus* metacestodes, it has been found that ABZ and MBZ exhibit a parasitostatic rather than a parasitocidal effect in human patients suffering from AE [35]. Therefore, recurrence rates after interruption of therapy are high in these last cases. Nevertheless, chemotherapy has significantly increased the 10-year survival rate of inoperable or non-radically operated AE patients from 6–25% to 80–83% [7, 37].

Indeed, a major issue for patients with AE is the longevity after ABZ treatment and the dilemma as to when treatment can be stopped since there is no method currently available to determine when this decision should be taken [11].

Liver transplantation may also be indicated for highly selected patients with life-threatening complications after failure of other treatments [38].

4. DRUGS AGAINST *ECHINOCOCCUS SP*: MODE OF ACTION

4.1. BZ Mode of Action

The primary mode of action of BZ is an interaction with the eukaryotic cytoskeletal protein, b-tubulin, inhibiting its polymerization into microtubules [14]. As a secondary effect glucose uptake is reduced, leading to depletion of glycogen storage, degenerative alterations in the endoplasmic reticulum and mitochondria of the germinal layer and, finally, to cellular autolysis. Tubulin is a ubiquitous protein, present in the mammalian host as well as in the helminthic parasite, but the BZ bind selectively to parasite tubulin and, thus, have minor or no toxicity in mammals.

When *in vitro* cultures of metacestodes were treated with ABZ, microtubular ultrastructure was affected [39, 40] suggesting that ABZ interfered with tubulin polymerization [41]. Molecular genetics revealed that sensitivity to BZ in evolutionary distant organisms such as fungi, nematodes, platyhelmithes, and various protozoa was correlated with the presence of specific alleles of b-tubulin genes [23, 42-45].

4.2. Possible Mode of Action of PZQ in Hydatid Disease

PZQ does not penetrate well into the mature hydatid cyst and therefore does not inhibit cyst growth, but is a highly effective protoscolecidal agent both *in vitro* and *in vivo* and shows activity against early cysts as described previously. In contrast, ABZ is rapidly converted to an active metabolite, albendazole sulphoxide (ABZ-SO), which achieves high concentrations in the cyst and is active against both protoscoleces and the germinal membrane. The action of ABZ is the inhibition of microtubule assembly, with detected alterations in cyst tissue also occurring in the germinal layer [46]. It may be feasible that prior cyst wall damage by ABZ facilitates the penetration and action of PZQ [47].

5. NOVEL CHEMOTHERAPEUTICAL OPTIONS

In order to identify novel potential alternatives for chemotherapy against echinococcosis, the strategies most commonly used have been testing *in vitro* the agent on *E. multilocularis* and E. *granulosis* metacestodes and/or protoscoleces, and/or applying *in vivo* rodent models. In most cases, researchers have focused on broad-spectrum antiinfective agents and anti-cancer compounds, many of which also exhibited reasonable efficacy against *Echinococcus* [48]. Tested compounds include benzimidazole derivatives such as flubendazole, and oxfendazole [49-52]. Ceballos *et al.* [53] reported an excellent efficacy of FLBZ against a secondary hydatid disease developed in mice, even superior than that observed for ABZ under the same experimental conditions. Other anti-infective agents like ivermectin, nitazoxanide, genistein, artemisinin, timol, and rapamycin were also studied against *Echinococcus* [54-59]. Although some of these compounds showed promising *in vitro* activities, and to some extent also in the rodent models, they have not been translated into clinical applications.

Moreover, several drugs inhibiting proliferation of cancer cells were assayed on *Echinococcus* metacestodes and protoscoleces. Spicher *et al.* [60] demonstrated the *in vitro* and *in vivo* effects of an endogenous metabolite of estrogen, 2-methoxyestradiol, either alone or combined with ABZ, against *E. multilocularis* and *E. granulosus*. The cytostatic drug, imatinib, had drastic effects on the *in vitro* development and survival of *E. multilocularis* [61].

On the other hand, a problem that has emerged with the use of synthetic anthelmintics is the development of parasitic resistance, which can threaten the success of treatment in humans [62, 63]. Consequently, the search of new therapeutic alternatives such as the use of traditional medicinal plants has been increased [64]. The pharmaceutical properties of aromatic plants are partially attributed to essential oils [65]. Essential oils are volatile, natural, complex compounds characterized by a strong odour and formed by aromatic plants as secondary metabolites. These substances may be constituted for about 20-60 components at quite different concentrations. Therefore, the biological effects of essential oils may be the result of a synergism of all these molecules or reflect only those of the main molecules present at the

highest levels according to gas chromatographic analysis [66].

Because of its hydrophobic character, the essential oils or their components present a great potential of pharmacological applications as antimicrobial agents [65, 67, 68]. The role of essential oils against parasitic helminths has been studied by several authors [64, 69-71]. However, there are few publications about the effect of these substances on *E. granulosus*. The *in vitro* effect of the essential oils of *Rosmarinus officinalis* (rosemary), *Mentha pulegium*, *M. piperita*, *Pistacia khinjuk* (pistachio) and *Trachyspermum ammi* (ajowan) was demonstrated against protoscoleces of *E. granulosus* [72-75]. Moreover, the *in vitro* effect of thymol was observed against protoscoleces, microcysts and cysts of *E. granulosus* [54, 76].

Recently, Wu *et al.* [77] demonstrated that pericyst may be a new pharmacological and therapeutic target for hydatid disease. Most parasitic cysts with calcified walls, often associated with degenerative content and decreased size of hydatid cysts, are biologically inactive and clinically silent. When calcification is extensive, death of the parasite is implied because the large calcified surface reduces the nutritional exchange between the cyst and the surrounding parenchyma. Thus, pharmacological modulation of calcification in pericysts may be a new therapeutic target for this disease.

6. DRUG DELIVERY SYSTEMS (DDS) FOR HYDATID DISEASE

The main purpose of using a DDS is, as implied, not only to deliver a biological active compound in a controlled way (time period and releasing rate) but also to maintain drug level in the body within therapeutic window [78].

The limited progress in improving the efficacy of the treatment in severe disease cases has suggested a growing need for a multidisciplinary approach to deliver the therapeutic agents to specific targets in tissues. The success of the chemotherapeutic treatment of hydatid disease is based on the drug ability to act on the germinal layer and on the protoscolices of the hydatid cyst interior at adequate concentrations for sufficient periods of time [79]. Some studies on the use of DDS are summarized in Table **1**.

In 1993, Liance *et al*. [80] tested the efficacy of doxorubicin (DOXO) loaded onto polyisohexylcyanoacrylate (PIHCA) nanoparticles on liver murine AE in mice. In this study, nanoparticles loaded with doses of DOXO far less than those used to inhibit hepatic sarcoma metastases [81] were efficient in inhibiting the hepatic larval growth, and reducing the parasite viability. Nevertheless, it would be interest to evaluate the effects of higher doses administered during a shorter period of time. Because atypical periparasitic tissue reactions occurred with either free or bounded DOXO, such experiments would include an histopathological study in order to determine the effects of the drug on liver samples taken far from the parasite. An evaluation of DOXO-loaded nanoparticles during the course of murine hepatic AE enhances the development of less toxic anthelminthic compound-loaded nanoparticles.

Drug targeting strategies could make the intravenous (i.v.) administration of ABZ possible and could increase its availability in the liver. The aim of the study developed by Rodrigues *et al.* [82] was the design of a parenteral formulation of ABZ using poly (D,L-Lactide) (PLA) nanoparticles as a biodegradable carrier in order to evaluate the potential of this new colloidal antiparasitic drug delivery system in mice with *E. multilocularis* infection. The efficiency of ABZ incorporation to PLA nanoparticles was over 97% at the final ABZ concentration of 0.2 mg/mL and the system was not toxic for peritoneal macrophages at ABZ concentrations 10- fold higher than those which were found to impair the viability of *E. granulosus* protoscoleces.

Table 1. Drug Delivery Systems Studied for the Treatment of Echinococcosis

System	Drug	References
Polyisohexylcyanoacrylate (PIHCA) nanoparticles.	DOXO	Liance *et al.* Int J Parasitol 1993; 23: 427-429.
Poly (D,L-Iactide) (PLA) nanoparticles	ABZ	Rodrigues *et al.* Int J Parasitol 1995; 25: 1437-1441.
Solution: 40% (w:w) Transcutol® in 1.2 pH buffer (KCl:HCl))	ABZ	Torrado *et al.* Int J Pharm 1997; 156(2): 181-7.
Spot-on	PZQ	Jenkins DJ, Romig T. Int J Parasitol 2000; 30: 959-962.
30% soybean oil emulsion	ABZ	Mingjie *et al.* Acta Trop 2002; 83: 177-181
Emulsion: 30% soybean oil (v/v).	ABZ	Shuhua *et al.* Acta Trop 2002; 82:77-84.
Liposomes	ABZ	Dvoroznakova *et al.* Parasitol Int 2004; 53: 315-325.
Chitosan Microspheres	ABZ	Leonardi *et al.* J Pharm Biomed Anal 2008; 48: 802-807.
d,l,Polylactide nanoparticles	ABZ and/or ABZ-SO	Truong Cong, *et al.* Int J Pharm 2008; 353: 223-232.
Poly(ε-caprolactone) implants	PZQ	Cheng *et al.* Int J Pharm 2009; 377: 112-119.
Solid lipid nanoparticles	ABZ-SO	Ahmadnia *et al.* Exp Parasitol 2013; 135:314-319.

Interestingly, the absence of hepatic lesions in some ABZ-loaded nanoparticle-treated mice suggests that this formulation has increased the hepatic availability of the drug. Nevertheless, the failure to inhibit the metacestode establishment by prophylactic treatment still need to be explained.

As the ABZ therapy is specially important in systemic cestode infections, particularly in inoperable or disseminated cases of echinococcosis [83] and neurocysticercosis [84], different efforts have been made to improve ABZ solubility. Torrado *et al* [85] investigated a new liquid formulation, which consisted of a solvent system of Transcutol® (40% w:w) in 1.2 pH buffer, in order to improve the oral bioavailability of ABZ. The higher concentration–time plasma profile of the ABZ solution would explain the greater therapeutic effect obtained with this new formulation against the systemic phases of the *Trichinella* model studied. This ABZ solution presented an efficacy against the migrating larvae phase twice bigger than that of the ABZ suspension formulation. The ABZ solution presents a very high efficacy (95.5% reduction at 50 mg/kg dose) which may be specially important for inoperable or disseminated cases of other systemic cestode infections such as hydatidosis or neurocysticercosis.

With this purpose and following with the development of liquid formulations, Jenkins and Romig (2000) [86] have reported the results of an assessment of the efficacy of a "spot-on" dermal anthelmintic treatment containing 4% w/v PZQ against immature and mature *E. multilocularis* in cats. Thirty purpose-bred cats were experimentally infected each with 10 000 protoscoleces of *E. multilocularis*. Ten days later one group of ten cats was treated with Droncit Spot-on (PZQ) 4% w/v dermally in one place on the dorsal aspect of the neck at a dose of 8 mg/kg. Eleven days later (21 days p.i.) a second group of ten cats was also treated with DroncitR Spot-on the same way.

This study indicates that Droncit Spot-on (PZQ) 4% w/v applied dermally at 8 mg/kg is highly effective in removing *E. multilocularis* from the small intestine of cats infected with immature and mature (prepatent) infections of *E. multilocularis*. In the cats with the mature infections all tapeworms were absent from the small intestine within 2 days of treatment. It is worth to point out that nowadays, part of the preventative strategies against the transmission of human AE includes regular anthelminthic treatment of cats (and dogs) against tapeworms.

Nevertheless these results, ABZ is now considered as the chemotherapeutic treatment of choice for human echinococcosis. Based on almost two decades of experience, the cure rate for human cystic echinococcosis with ABZ is estimated to range between 20 and 40% [87]. In order to improve its therapeutic efficacy for use in China, Mingjie *et al*. [88] have formulated ABZ in a 30% soybean oil emulsion (AbzE).

Based on the apparent success of treating murine echinococcosis with AbzE [89] undertook an initial evaluation of this agent in humans with *E. granulosus* infection. Either AbzE or Abz tablets (AbzT) was administered at a dose corresponding to 12.5 mg/kg of the ABZ component (maximal dose of 1 g) to seven male patients diagnosed with CE. For the initial dose, AbzT was administered to each patient approximately 2 h after the first morning meal. Both Abz absorption and serum levels of ABZ-SO were increased following AbzE administration relative to AbzT administration. Due to its significant improvement of bioavailability, AbzE will be recommended for evaluation in the clinical treatment of echinococcosis [89], although striking individual differences in the area under the curve (AUC) levels were seen after oral administration of AbzT and AbzE, particularly in AbzE group.

In another attempt to improve the ABZ therapy, Shuhua *et al*. [90] developed and characterized a new drug emulsified system containing 30% of soybean oil. The emulsion was more effective than ABZ solution in reducing the weight of hydatid cysts in mice.

Furthermore, Dvorožňáková *et al*. [91] studied the effect of the treatment with free and liposomized ABZ on selected immunological parameters and cyst growth in mice infected with *E. multilocularis*. The size-distribution of vesicles was approximately 73.1% of the total number of particles ranged between 2.02 and 4.76 μm. The concentration of ABZ in liposomes was 3.07 mg/mL and the total phospholipid content was 12.75 mg/ml. The significant reduction of cyst weight in comparison with untreated mice ($P<0.01$) was recorded after the last drug dose from week 10 up to week 14 p.i. While in the ABZ-treated group decreased cyst growth was seen for 4 weeks after the termination of therapy, administration of ABZ in liposomes significantly ($P<0.05$) extended the parasitostatic effect of the drug for 4 more weeks. However, the complete inhibition of cyst growth was not achieved under either chosen drug regime. Untreated mice were in a poor condition of health from week 22 p.i.

The same study demonstrates that administration of both free ABZ and ABZ incorporated in liposomes significantly modifies selected immunoregulatory and cytotoxic components of cellular immunity in experimental AE. These findings indicate that anthelmintic potency of ABZ can be increased after incorporation into liposomes not only because of improved pharmacokinetics and consequent bioavailability, but also because of significant stimulation of Th1-type cytokine IFN-γ response and effector macrophage functions.

Leonardi *et al*. [92] developed ABZ–chitosan microspheres with high dissolution rate and encapsulation efficiency in order to obtain a systemic action for the treatment of different hydatidic cysts. Several factors in the particle formulation were evaluated to distinguish those which have a significant effect on six responses: yield, pH, morphology, size, dissolution rate and encapsulation efficiency of the microparticles. The dissolution profiles for a formulation obtained in the selected conditions were contrasted against ABZ without any treatment. The microparticles formulation showed an enhanced dissolution rate for ABZ, in comparison to the drug alone.

Recently, Truong Cong *et al*. [79] formulated BZ-loaded nanoparticles to increase the apparent solubility of the drug and to avoid its hepatic metabolism. The particles containing ABZ and ABZ-SO were obtained by an emulsion solvent evaporation method using PLA as carrier. The size of the

particles was around 200 nm. In this work the authors performed an ex vivo permeation study through hydatid cyst membranes. The permeation coefficient correlated closely with the drug partition coefficient log P ($r = 0.951$, $P < 0.05$). From these results, it was possible to propose a simple model to determine the molecule diffusion through the hydatid cyst membrane. Thus, the permeation coefficient, P, could be estimated from the partition coefficient, log P. In fact, these findings confirmed the preponderant role played by the lipophilicity of the anthelmintic drugs and the barrier function of the cyst germinal layer allowing the design of new drug delivery systems taking into account this premise.

Wei *et al.* [93] developed a long-term sustained release implantable PZQ-containing bar, and later Cheng *et al.* [94] prepared a PZQ-loaded sustained-release Poly(ε-caprolactone) (PCL) implant and characterized them in terms of drug thermal stability during the preparation process, content uniformity, physical state of drug and implant stability under storage condition. The implants were prepared by fully blending PZQ particles with melting PCL at different ratios and then molding the blends into cylindrical implants by a lab-scale injection molder. PZQ was stable during the fabrication process. The surfaces of all implants were smooth and devoid of any pores and cracks. The *in vitro* drug release was also evaluated. The systems shown a burst release that could be ascribed to immediate dissolution of the drug located on or near the surface of implants after immersion in the release medium. Following to this, PZQ release rate gradually slowed down and tended to be a constant regime. This type of devices can be an interesting technological alternative for the hydatid treatment.

In 2013, Ahmadnia *et al*. [95] published a very interesting work related to the evaluation of the efficacy of ABZ and ABZ-SO loaded solid lipid nanoparticles (SLN). ABZ-SO loaded into SLN have been produced for the first time using the solvent diffusion evaporation technique. The mean particle size and size distribution of SLN and ABZ-SO loaded SLN were 370±140 and 380±125 nm, respectively. The SLNs were stable in 2–8 °C for at least 90 days but their stability were limited at room temperature. The entrapment efficiency was assessed 78% and zeta potential of SLNs was −2.41 mV. Hydatid cyst developed in 94% of the infected animals. Treatment of mice with ABZ and ABZ-SO loaded SLN with the dose of 0.5 mg/kg and with the dose of 2 mg/kg every 48 h resulted in reduction of the cysts size and weight compared to the control groups, however, the corresponding reductions were not statistically significant. These results should be supported by further biochemical and molecular studies before introducing this treatment as an efficient therapeutic regimen in treating hydatidosis in humans and animals but are a good starting point for the use of lipid systems in the treatment of human echinococcosis.

7. PERSPECTIVES

Modern developments in the science of pharmacology are based on improved knowledge of basic mechanisms of drug action and of the molecular basis of disease, together with an explosion of technological advances in multiple fields, including analytical chemistry, computational sciences, molecular biology, genomics and material engineering. It is the projection of these transforming technologies onto our current knowledge base that provides a realistic approach to predicting what the discipline may well resemble in the future.

Surveillance of human CE is critical in order to measure the public health impact of any hydatid control program. Also, a detailed knowledge of the life cycle of the parasite is important in relation to rational implementation of hydatid control programs.

Recently, joint and coordinated implementation of these health measures, both medical and veterinary, has resulted in noteworthy improvement in the results of the control campaigns.

The responsibility for educating the public belongs to all health professionals, whether engaged in private or public practice. The success of hydatidosis eradication programs around the world illustrates the value of educating the public to accomplish goals.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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REFERENCES

[1] Chomel BB. Control and prevention of emerging parasitic zoonoses. Int J Parasitol 2008; 38: 1211-1217.

[2] Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. Helminth infections: the great neglected tropical diseases. J Clinl Invest 2008; 118: 1311-1321.

[3] WHO. Neglected tropical diseases. http://www.who.int/neglecteddiseases/zoonoses/en/ (2012).

[4] McManus DP, Gray DJ, Zhang W, Yang Y. Diagnosis, treatment, and management of echinococcosis. BMJ 2012; 344: e3866.

[5] Budke CM, Deplazes P, Torgerson PR. Global socioeconomic impact of cystic echinococcosis. Emerg Infect Dis 2006; 12: 296-303.

[6] Torgerson PR, Keller K, Magnotta M, Ragland N. The global burden of alveolar echinococcosis. PLoS Negl Trop Dis 2010; 4: e722.

[7] Eckert J, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin Microbiol Rev 2004; 17: 107-135.

[8] Brunetti E, White AC, Jr. Cestode infestations: hydatid disease and cysticercosis. Infect Dis Clin North Am 2012; 26: 421-435.

[9] Thompson RC. The taxonomy, phylogeny and transmission of Echinococcus. Exp Parasitol 2008; 119: 439-446.

[10] Craig PS, McManus DP, Lightowlers MW, *et al.* Prevention and control of cystic echinococcosis. Lancet Infect Dis 2007; 7: 385-394.

[11] McManus DP. Echinococcosis with particular reference to Southeast Asia. Adv Parasitol 2010; 72: 267-303.

[12] Eckert J. In: The biology of *Echinococcus* and hydatid disease; Thompson RCA, Ed. London, United Kingdom 1986 pp. 250–284.

[13] Pawlowski ZS, Eckert J, Vuitton DA, *et al.* In: WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern World Organisation for Animal Health; Eckert J, Gemmell MA, Meslin F, Pawlowski ZS, Eds. Paris, France 2001 pp. 20–66.

[14] Kern P. *Echinococcus granulosus* infection: clinical presentation, medical treatment and outcome. Langenbecks Arch Surg 2003; 388: 413-20.

[15] Kern P. Medical treatment of echinococcosis under the guidance of Good Clinical Practice (GCP/ICH). Parasitol Int 2006; 55: S273-S282.

[16] Dervenis C, Delis S, Avgerinos C, Madariaga J, Milicevic M. Changing concepts in the management of liver hydatid disease. J Gastrointest Surg 2005; 9:869-877.

[17] WHO-IWGE. WHO-Informal Working Group on Echinococcosis. Guidelines for treatment of cystic and alveolar echinococcosis in humans. Bull World Health Organ 1996; 74:231-242.

[18] Stey C, Jost R, Ammann R. Rezidivierende, Lebensbedrohliche Anaphylaxie Als Erstmanifestation Eines *Echinococcus cysticus* (*granulosus*) Der Leber. Schweiz Med Wochenschr Suppl 1993; 123: 1445-1450.

[19] Pawlowski ZS. In:. Compendium on cystic echinococcosis; Anderson FL, Ouhelli H, Kachani M, Eds. Brigham Young University, Provo 1997; pp. 119-135.

[20] Mandal S, Mandal MD. Human cystic echinococcosis: epidemiologic, zoonotic, clinical, diagnostic and therapeutic aspects. Asian Pac J Trop Med 2012; 5: 253-260.

[21] Ormeci N, Idilman R, Akyar S, *et al*. Hydatid cysts in muscle: a modified percutaneous treatment approach. Int J Infec Dis 2007;11: 204-208.

[22] Neumayr A, Troia G, de Bernardis C, *et al*. Justified concern or exaggerated fear: the risk of anaphylaxis in percutaneous treatment of cystic echinococcosis-a systematic literature review. PLoS Negl Trop Dis 2011; 5:e1154.

[23] Hemphill A, Muller J. Alveolar and cystic echinococcosis: towards novel chemotherapeutical treatment options. J Helminthol 2009; 83: 99-111.

[24] Ceballos L, Elissondo C, Moreno L, *et al*. Albendazole treatment in cystic echinococcosis: pharmacokinetics and clinical efficacy of two different aqueous formulations. Parasitol Res 2008; 103:355-362.

[25] Moro P, Schantz PM. Echinococcosis: a review. Inter J Infec Dis 2009; 13:125-133.

[26] Horton RJ. Albendazole in treatment of human cystic echinococcosis: 12 years of experience. Acta Trop 1997; 64:79-93.

[27] Horton J. Albendazole for the treatment of echinococcosis. Fundam Clin Pharmacol 2003; 17:205-212.

[28] Zhang X-N, Tang L-H, Gong J-H, Xu X-Y, Zhang Q. Alternative Albendazole Polybutylcyanoacrylate Nanoparticles Preparation, Pharmaceutical Properties and Tissue Distribution in Rats. Lett Drug Des Discov 2006; 3:275-280.

[29] King CH. In: Principles and Practice of Infectious Diseases; Mandell GL, Bennett JE, Dolin R, Eds. Saunders: Philadelphia 2005; pp. 3290-93.

[30] Vuitton DA. Benzimidazoles for the treatment of cystic and alveolar echinococcosis: what is the consensus? Expert Rev Anti Infect Ther 2009; 7: 145-9.

[31] Urrea-Paris MA, Casado N, Moreno MJ, Rodriguez-Caabeiro F. Chemoprophylactic praziquantel treatment in experimental hydatidosis. Parasitol Res 2001; 87: 510-512.

[32] Cobo F, Yarnoz C, Sesma B, *et al*. Albendazole plus praziquantel versus albendazole alone as a pre-operative treatment in intra-abdominal hydatidosis caused by *Echinococcus granulosus*. Trop Med Int Health 1998; 3: 462-466.

[33] Brunetti E, Kern P, Vuitton DA, Writing Panel for the WHO-IWGE. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop 2010; 114:1-16.

[34] Eckert J, Schantz PM, Gasser RB, *et al*. In: WHOI/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern World Organisation for Animal Health; Eckert J, Gemmell MA, Meslin F-X, Pawlowski ZS, Eds. Paris, France 2001; pp. 101-143.

[35] Reuter S, Buck A, Manfras B, *et al*. Structured treatment interruption in patients with alveolar echinococcosis. Hepatol 2004; 39: 509-517.

[36] Reuter S, Jensen B, Buttenschoen K, Kratzer W, Kern P. Benzimidazoles in the treatment of alveolar echinococcosis: a comparative study and review of the literature. J Antimicrob Chemother 2000; 46: 451-456.

[37] Ammann R, Eckert JI. In: Echinococcosis and Hydatid Disease; Thompson RC, Lymbery AJ, Eds. CAB International; Wallmingford 1995; pp. 411-430.

[38] Mantion G, Bresson-Hadni S, Vuitton DA, Wen H, Chapuis Y, Gillet M. Treatment of alveolar echinococcosis: a multidisciplinary task. Bull Acad Natl Med 2008; 192:1151-1157.

[39] Ingold K, Bigler P, Thormann W, Cavaliero T, Gottstein B, Hemphill A. Efficacy of albendazole sulfoxide and albendazole sulfone against *In vitro*-cultivated *Echinococcus multilocularis* metacestodes. Antimicrob Agents Chemother 1999; 43: 1052-1061.

[40] Rubino S, Fiori PL, Lubinu G, Monaco G, Cappuccinelli P. The cytoskeleton of hydatid cyst cultured cells and its sensitivity to inhibitors. Eur J Cell Biol 1983; 30: 182-190.

[41] Lacey E. Mode of action of benzimidazoles. Parasitol Today 1990; 6:112-115.

[42] Driscoll M, Dean E, Reilly E, Bergholz E, Chalfie M. Genetic and molecular analysis of a *Caenorhabditis elegans* beta-tubulin that conveys benzimidazole sensitivity. J Cell Biol 1989; 109: 2993-3003.

[43] Henriquez FL, Ingram PR, Muench SP, Rice DW, Roberts CW. Molecular basis for resistance of *Acanthamoeba tubulins* to all major classes of antitubulin compounds. Antimicrob Agents Chemother 2008; 52: 1133-1135.

[44] Katiyar SK, Gordon VR, McLaughlin GL, Edlind TD. Antiprotozoal activities of benzimidazoles and correlations with beta-tubulin sequence. Antimicrob Agents Chemother 1994; 38: 2086-2090.

[45] Kwa MS, Veenstra JG, Van Dijk M, Roos MH. Beta-tubulin genes from the parasitic nematode *Haemonchus contortus* modulate drug resistance in *Caenorhabditis elegans*. J Mol Biol 1995; 246: 500-510.

[46] Casado N, Perez-Serrano J, Denegri G, Rodriguez-Caabeiro F. Development of chemotherapeutic model for the *in vitro* screening of drugs against *Echinoccus granulosus* cysts: the effects of an albendazole-albendazole sulphoxide combination. Int J Parasitol 1996; 26: 59-65.

[47] Bygott JM, Chiodini PL. Praziquantel: Neglected drug? Ineffective treatment? Or therapeutic choice in cystic hydatid disease? Acta Trop 2009; 111: 95-101.

[48] Hemphill A, Stadelmann B, Scholl S, *et al*. *Echinococcus* metacestodes as laboratory models for the screening of drugs against cestodes and trematodes. Parasitol 2010; 137: 569-587.

[49] Elissondo M, Dopchiz M, Ceballos L, *et al*. *In vitro* effects of flubendazole on *Echinococcus* granulosus protoscoleces. Parasitol Res 2006; 98: 317-323.

[50] Elissondo M, Ceballos L, Dopchiz M, *et al*. *In vitro* and *in vivo* effects of flubendazole on *Echinococcus granulosus* metacestodes. Parasitol Res 2007; 100: 1003-1009..

[51] Ceballos L, Elissondo M, Bruni SS, Denegri G, Alvarez L, Lanusse C. Flubendazole in cystic echinococcosis therapy: pharmaco-parasitological evaluation in mice. Parasitol Int 2009; 58: 354-358.

[52] Gavidia CM, Gonzalez AE, Lopera L, *et al*. Evaluation of nitazoxanide and oxfendazole efficacy against cystic echinococcosis in naturally infected sheep. Am J Trop Med Hyg 2009; 80: 367-372.

[53] Ceballos L, Elissondo C, Sanchez Bruni S, Denegri G, Lanusse C, Alvarez L. Comparative performances of flubendazole and albendazole in cystic echinococcosis: *ex vivo* activity, plasma/cyst disposition, and efficacy in infected mice. Antimicrob Agents Chemother 2011; 55: 5861-5867.

[54] Elissondo MC, Albani CM, Gende L, Eguaras M, Denegri G. Efficacy of thymol against *Echinococcus granulosus* protoscoleces. Parasitol Int 2008; 57: 185-190.

[55] Elissondo MC, Ceballos L, Alvarez L, Sanchez Bruni S, Lanusse C, Denegri G. Flubendazole and ivermectin *in vitro* combination therapy produces a marked effect on *Echinococcus granulosus* protoscoleces and metacestodes. Parasitol Res 2009; 105: 835-842.

[56] Walker M, Rossignol JF, Torgerson P, Hemphill A. *In vitro* effects of nitazoxanide on *Echinococcus granulosus* protoscoleces and metacestodes. J Antimicrob Chemother 2004; 54: 609-616.

[57] Naguleswaran A, Spicher M, Vonlaufen N, *et al*. *In vitro* metacestodicidal activities of genistein and other isoflavones against *Echinococcus multilocularis* and *Echinococcus granulosus*. Antimicrob Agents Chemother 2006; 50: 3770-3778.

[58] Spicher M, Naguleswaran A, Ortega-Mora LM, Muller J, Gottstein B, Hemphill A. *In vitro* and *in vivo* effects of 2-methoxyestradiol, either alone or combined with albendazole, against *Echinococcus* metacestodes. Exp Parasitol 2008; 119: 475-482.

[59] Cumino AC, Lamenza P, Denegri GM. Identification of functional FKB protein in *Echinococcus granulosus*: its involvement in the protoscolicidal action of rapamycin derivates and in calcium homeostasis. Int J Parasitol 2010; 40: 651-661.

[60] Spicher M, Roethlisberger C, Lany C, *et al*. *In vitro* and *in vivo* treatments of echinococcus protoscoleces and metacestodes with

artemisinin and artemisinin derivatives. Antimicrob Agents Chemother 2008; 52: 3447-3450.

[61] Hemer S, Brehm K. *In vitro* efficacy of the anticancer drug imatinib on *Echinococcus multilocularis* larvae. Int J Antimicrob Agents 2012; 40: 458-462.

[62] Doenhoff MJ, Cioli D, Utzinger J. Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. Curr Opin Infect Dis 2008; 21: 659-667.

[63] Fenwick A, Webster JP. Schistosomiasis: challenges for control, treatment and drug resistance. Curr Opin Infec Dis 2006; 19: 577-582.

[64] Jeyathilakan N, Murali K, Anandraj A, Latha B, Abdul B. Anthelmintic activity of essential oils of *Cymbopogon nardus* and *Azadirachta indica* on *Fasciola gigantica*. J Vet Animal Sci. 2010; 6: 204-209.

[65] Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytother Res 2007; 21: 308-323.

[66] Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils-a review. Food Chem Toxicol 2008; 46: 446-475.

[67] Arana-Sanchez A, Estarron-Espinosa M, Obledo-Vazquez EN, Padilla-Camberos E, Silva-Vazquez R, Lugo-Cervantes E. Antimicrobial and antioxidant activities of Mexican oregano essential oils (*Lippia graveolens* H. B. K.) with different composition when microencapsulated in beta-cyclodextrin. Lett Appl Microbiol 20101; 50: 585-590.

[68] Machado M, Dinis AM, Salgueiro L, Cavaleiro C, Custodio JB, Sousa Mdo C. Anti-Giardia activity of phenolic-rich essential oils: effects of *Thymbra capitata*, *Origanum virens*, *Thymus zygis* subsp. *sylvestris*, and *Lippia graveolens* on trophozoites growth, viability, adherence, and ultrastructure. Parasitol Res 2010; 106: 1205-1215.

[69] Camurca-Vasconcelos AL, Bevilaqua CM, Morais SM, *et al*. Anthelmintic activity of *Croton zehntneri* and *Lippia sidoides* essential oils. Vet Parasitol 2007; 148: 288-294.

[70] Githiori JB, Athanasiadou S, Thamsborg SM. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. Vet Parasitol 2006; 139: 308-320.

[71] Macedo IT, Bevilaqua CM, de Oliveira LM, *et al.* Anthelmintic effect of *Eucalyptus staigeriana* essential oil against goat gastrointestinal nematodes. Vet Parasitol 2010; 173: 93-98.

[72] Albanese A, Elissondo M, Gende L, Eguaras M, Denegri M. *Echinococcus granulosus*: *in vitro* efficacy of *Rosmarinus officinalis* essential oil on protoscoleces. Int J Essen Oil Ther 2009; 3n:69-75.

[73] Maggiore MA, Albanese AA, Gende LB, Eguaras MJ, Denegri GM, Elissondo MC. Anthelmintic effect of *Mentha* spp. essential oils on *Echinococcus granulosus* protoscoleces and metacestodes. Parasitol Res 2012; 110:1103-1112.

[74] Moazeni M, Saharkhiz MJ, Hosseini AA. *In vitro* lethal effect of ajowan (*Trachyspermum ammi* L.) essential oil on hydatid cyst protoscoleces. Vet Parasitol 2012; 187: 203-208.

[75] Taran M, Azizi E, Shikhvaisi A, Asadi N. The anthelmintic effect of *Pistacia khinjuk* against protoscoleces of *Echinococcus granulosus*. World J Zoo 2009; 4: 291-295.

[76] Elissondo MC, Pensel PE, Denegri GM. Could thymol have effectiveness on scolices and germinal layer of hydatid cysts? Acta Trop 2013; 125:251-257.

[77] Wu XW, Chen XL, Zhang SJ, Zhang X, Sun H, Peng XY. Pericyst may be a new pharmacological and therapeutic target for hydatid disease. Chin Med J 2011; 124: 2857-2862.

[78] Coelho JF, Ferreira PC, Alves P, *et al*. Drug delivery systems: Advanced technologies potentially applicable in personalized treatments. EPMA J 2010; 1: 164-209.

[79] Truong Cong T, Faivre V, Nguyen TT, *et al*. Study on the hydatid cyst membrane: permeation of model molecules and interactions with drug-loaded nanoparticles. Int J Pharm 2008; 353: 223-232.

[80] Liance M, Nemati F, Bories C, Couvreur P. Experience with doxorubicin-bound polyisohexylcyanoacrylate nanoparticles on murine alveolar echinococcosis of the liver. Int J Parasitol 1993; 23: 427-429.

[81] Chiannilkulchai N, Driouich Z, Benoit JP, Parodi AL, Couvreur P. Nanoparticles of doxorubicin: colloidal vectors in the treatment of hepatic metastases in animals. Bull Cancer 1989; 76: 845-848.

[82] Rodrigues JM, Jr., Bories C, Emery I, Fessi H, Devissaguet JP, Liance M. Development of an injectable formulation of albendazole and *in vivo* evaluation of its efficacy against *Echinococcus multilocularis* metacestode. Int J Parasitol 1995; 25: 1437-1441.

[83] Wen H, New RR, Craig PS. Diagnosis and treatment of human hydatidosis. Br J Clin Pharm 1993; 35:565-574.

[84] Del Brutto OH, Sotelo J, Roman GC. Therapy for neuro-cysticercosis: a reappraisal. Clin Infec Dis 1993; 17: 730-735.

[85] Torrado S, López ML, Torrado G, Bolás F, Torrado S, Cadórniga R. A novel formulation of albendazole solution: oral bioavailability and efficacy evaluation. Int J Pharm 1997; 156: 181-187.

[86] Jenkins DJ, Romig T. Efficacy of Droncit Spot-on (praziquantel) 4% w/v against immature and mature *Echinococcus multilocularis* in cats. Int J Parasitol 2000; 30: 959-962.

[87] Nahmias J, Goldsmith R, Soibelman M, el-On J. Three- to 7-year follow-up after albendazole treatment of 68 patients with cystic echinococcosis (hydatid disease). Ann Trop Med Parasitol 1994; 88: 295-304.

[88] Mingjie W, Shuhua X, Junjie C, *et al.* Albendazole-soybean oil emulsion for the treatment of human cystic echinococcosis: evaluation of bioavailability and bioequivalence. Acta Trop 2002; 83: 177-181.

[89] Chai J, Menghebat, Jiao W, Sun D, *et al.* Clinical efficacy of albendazole emulsion in treatment of 212 cases of liver cystic hydatidosis. Chin Med J 2002; 115: 1809-1813.

[90] Shuhua X, Jiqing Y, Mingjie W, *et al.* Augmented bioavailability and cysticidal activity of albendazole reformulated in soybean emulsion in mice infected with *Echinococcus granulosus* or *Echinococcus multilocularis.* Acta tropica 2002; 82: 77-84.

[91] Dvoroznakova E, Hrckova G, Boroskova Z, Velebny S, Dubinsky P. Effect of treatment with free and liposomized albendazole on selected immunological parameters and cyst growth in mice infected with *Echinococcus multilocularis.* Parasitol Int 2004; 53: 315-325.

[92] Leonardi D, Lamas MC, Olivieri AC. Multiresponse optimization of the properties of albendazole-chitosan microparticles. J Pharm Biomed Anal 2008; 48: 802-807.

[93] Wei J, Cheng F, Qun Q, *et al.* Epidemiological evaluations of the efficacy of slow-released praziquantel-medicated bars for dogs in the prevention and control of cystic echinococcosis in man and animals. Parasitol Int 2005; 54: 231-236.

[94] Cheng L, Guo S, Wu W. Characterization and *in vitro* release of praziquantel from poly(epsilon-caprolactone) implants. Int J Pharm 2009; 377: 112-119.

[95] Ahmadnia S, Moazeni M, Mohammadi-Samani S, Oryan A. *In vivo* evaluation of the efficacy of albendazole sulfoxide and albendazole sulfoxide loaded solid lipid nanoparticles against hydatid cyst. Exp Parasitol 2013; 135: 314-319.

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