

In vitro efficacies of solubility-improved mebendazole derivatives against *Echinococcus multilocularis*

Research Article

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Author for correspondence:

Zhisheng Dang, E-mail: dangzs@nipd.chinacdc.cn and Yumin Zhao, E-mail: zymcsg@sina.com

Shuo Xu^{1,2,3,4,5}, Liping Duan^{2,3,4,5}, Haobing Zhang^{2,3,4,5}, Bin Xu^{2,3,4,5}, Junhu Chen^{2,3,4,5}, Wei Hu^{2,3,4,5}, Weifeng Gui¹, Fuqiang Huang⁶, Xu Wang⁷, Zhisheng Dang^{2,3,4,5} and Yumin Zhao¹

¹Department of Parasitology, School of Basic Medicine, Guilin Medical University, Guilin, Guangxi 541004, P.R. China; ²Key Laboratory on Biology of Parasite and Vector, Ministry of Health, Shanghai, China; ³National Center for International Research on Tropical Diseases, Shanghai, China; ⁴WHO Collaborating Center for Tropical Diseases, Shanghai, China; ⁵National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai 200025, P.R. China; ⁶School of Life Science and Engineering, Foshan University, Foshan 528231, P.R. China and ⁷Henan Science and Technology Exchange Center with Foreign Countries, Zhengzhou, Henan 450003, P.R. China

Abstract

Recently, we introduced an epoxy group to mebendazole by a reaction with epichlorohydrin and obtained two isoforms, mebendazole C1 (M-C1) and mebendazole C2 (M-C2). The *in vitro* effects of mebendazole derivatives at different concentrations on *Echinococcus multilocularis* protoscoleces and metacestodes as well as cytotoxicity in rat hepatoma (RH) cells were examined. The results demonstrated that the solubility of the two derivatives was greatly improved compared to mebendazole. The mortality of protoscoleces *in vitro* reached to 70–80% after 7 days of exposure to mebendazole or M-C2, and M-C2 showed higher parasitocidal effects than mebendazole ($P > 0.05$). The parasitocidal effect of M-C1 was low, even at a concentration of 30 μM . The percentage of damaged metacestodes that were treated with mebendazole and M-C2 *in vitro* at different concentrations were similar, and M-C1 exhibited insignificant effects on metacestodes. Significant morphological changes on protoscoleces and metacestodes were observed after treatment with mebendazole and M-C2. In addition, the introduction of an epoxy group to mebendazole also reduced its cytotoxicity in RH cells. Our results demonstrate that the introduction of an epoxy group not only improved the solubility of mebendazole, but also increased its parasitocidal effects on *E. multilocularis* and reduced its cytotoxicity in RH cells.

Introduction

Hydatid disease, or echinococcosis, is a zoonosis caused by infection with the larva of *Echinococcus* parasites in visceral organs of human beings and other animals. The disease has become a global public health problem and has reached epidemic proportions in China, especially in the Northwestern livestock farming region. Livestock farming is one of the main economic activities in the Northwestern region of China. The nomadic and semi-nomadic lifestyle is one of the most important risk factors for the prevalence of echinococcosis (Jiang, 2002). The increasing incidence of echinococcosis threatens the health and safety of farmers and herdsman in the region. Surgical lesion removal together with albendazole/mebendazole treatment is lifesaving (Li *et al.*, 2010). However, complete removal of lesions is very difficult and cases are often only diagnosed at advanced stages of the disease. Therefore, it is of great importance to develop more effective drugs to safeguard the life of farmers and herdsman, and thus promote the development of animal farming in the region.

Albendazole and mebendazole are the most important benzimidazole drugs that exhibit good efficacy and a wide range of parasitocidal activity against intestinal helminthes and other parasites (Meng *et al.*, 2009; Li and Zhang, 2013). These two drugs exhibit similar mechanisms, with which they bind to microtubulin and interfere with the normal assembly of microtubules, resulting in a metabolic disorder. Benzimidazole drugs are fatal for parasites, as they cause denaturation of cytoplasmic microtubules in intestinal cells, which interferes with intracellular transportation (Jasmer *et al.*, 2000; Mottier *et al.*, 2006). Mebendazole and albendazole are the only two drugs recommended by the WHO for echinococcosis treatment (Teggi *et al.*, 1993), even though they exhibit inadequate solubility and absorbance (bioavailability) in humans. Therefore, there is a need to make these drugs more effective by improving their solubility and thus bioavailability (Liu and Zhang, 2009).

To date, several new formulations have been developed. Phospholipid-based liposomes can be double-molecule carriers that bind the anti-echinococcosis drug albendazole (liposomal albendazole), which increases the fat solubility and stability of albendazole (Ao Qier *et al.*, 2003). In addition, great progress has been made in highly effective albendazole emulsions

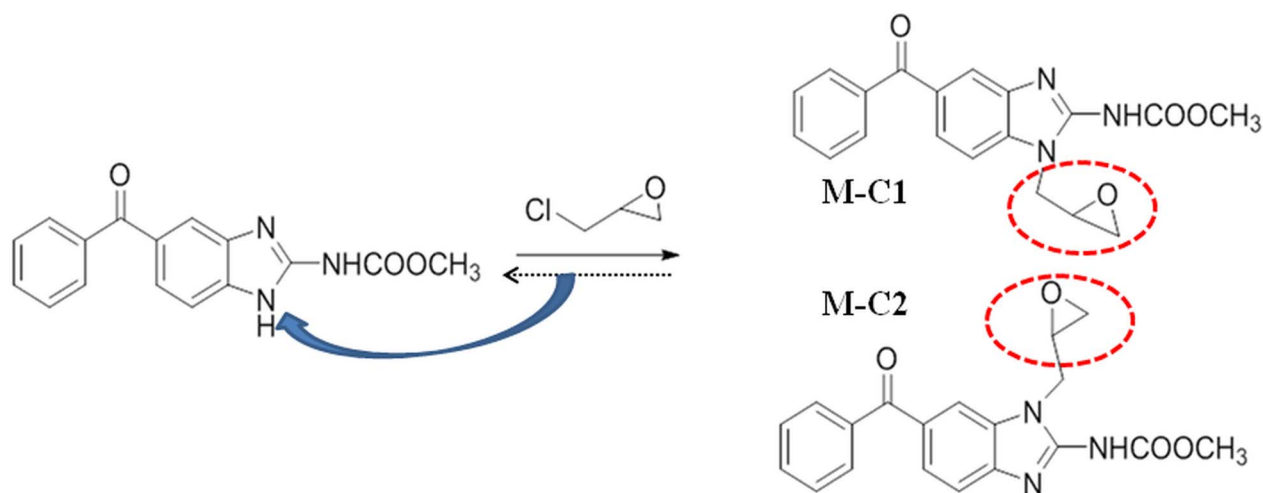


Fig. 1. (Colour online) Scheme of the synthesis of mebendazole compounds.

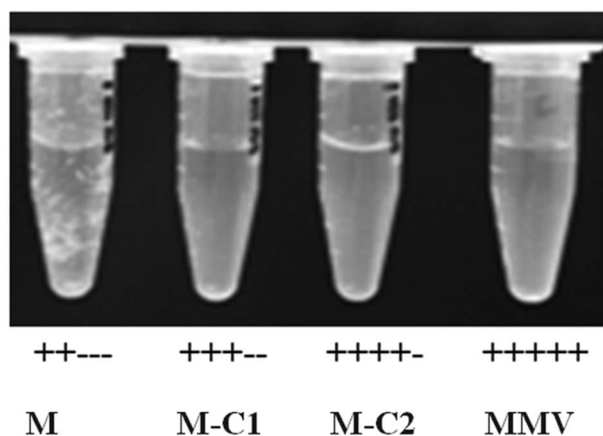


Fig. 2. Solubility of mebendazole compounds. M, mebendazole. Compounds were dissolved in DMSO-PBS (1:9) at a concentration of 1 mM. No visible particles or droplets were regarded as completely dissolved (scored as '+++++').

that exhibit increased absorption without increasing the toxicity of albendazole (Chai *et al.*, 2001).

Modification of certain groups is another way to increase benzimidazole solubility. A typical example is the introduction of a highly hydrosoluble phosphonooxymethyl group to triclabendazole (a benzimidazole derivative), which increases solubility 50 000-fold compared with triclabendazole and results in good faciolicidal activity *in vitro* and *in vivo* (Flores-Ramos *et al.*, 2014). Our present study has demonstrated that the introduction of an epoxy group to mebendazole not only improves its solubility but also increases its efficacy against *E. multilocularis* protoscoleces and metacestodes. This work offers new insights into developing novel benzimidazole derivatives against echinococcosis.

Materials and methods

Animal and parasite infection

Four-week-old female mice (Kunming strain) were purchased from SLAC Laboratory Animal Center (Shanghai, China). They were maintained in cages in a P2 animal room at 23–25 °C with a 12 h light/12 h dark cycle. Litter was cleaned weekly. They were provided with food and water *ad libitum*. An infected

mouse was sacrificed and protoscoleces (Qinghai strain) were purified and collected. The experimental mice were inoculated with 2000 protoscoleces intraperitoneally for about 3 months for block collection of protoscoleces and metacestodes.

Media and biochemical

Unless stated otherwise, all culture media and reagents were purchased from Gibco-BRL (New York, NY, USA) or Sigma (St. Louis, MO, USA).

Compound synthesis and solubility confirmation

The epoxy group was introduced to mebendazole by a reaction with epichlorohydrin and produced two isoforms, M-C1 and M-C2 (Fig. 1). Compounds were prepared at a concentration of 1 mM in 10% DMSO (dimethyl sulfoxide) solution [dissolved in PBS (phosphate buffered saline)] at 25 ± 2 °C and strongly shook for 30 s every 5 min. After 30 min, the solubility was assessed and was regarded as completely dissolved if there were no visible particles or droplets. The solubility degree was ranked with '+' and '-', where 5 '+' denoted complete dissolution (Fig. 2).

In vitro culture of *E. multilocularis* protoscoleces and metacestodes

Echinococcus multilocularis were cultured as previously described (Spiliotis and Brehm, 2009). In brief, metacestodes blocks from an infected KM mouse were cut and then crushed through a metal tea strainer. Metacestodes were washed three times with PBS. Protoscoleces were purified and stained with Trypan blue to confirm the survival rate (Liu *et al.*, 2015). Protoscoleces were cultured with 1066 medium [supplemented with 10% fetal bovine serum (FBS), 100 U mL⁻¹ penicillin G and 100 µg mL⁻¹ streptomycin sulphate]. Metacestodes were co-cultured with rat hepatocytes (5 × 10⁶) in DMEM medium (Dulbecco Modified Eagle's Medium, supplemented with 10% FBS, 100 U mL⁻¹ penicillin G and 100 µg mL⁻¹ streptomycin sulphate). Both protoscoleces and metacestodes were incubated in culture flasks at 37 °C, 5% CO₂ with medium changes once a week. The splitting of metacestod cultures was performed when the total metacestod volume exceeded 20 mL, and metacestodes were used for experimental procedures when they reached a diameter of 2–4 mm (Küster *et al.*, 2011).

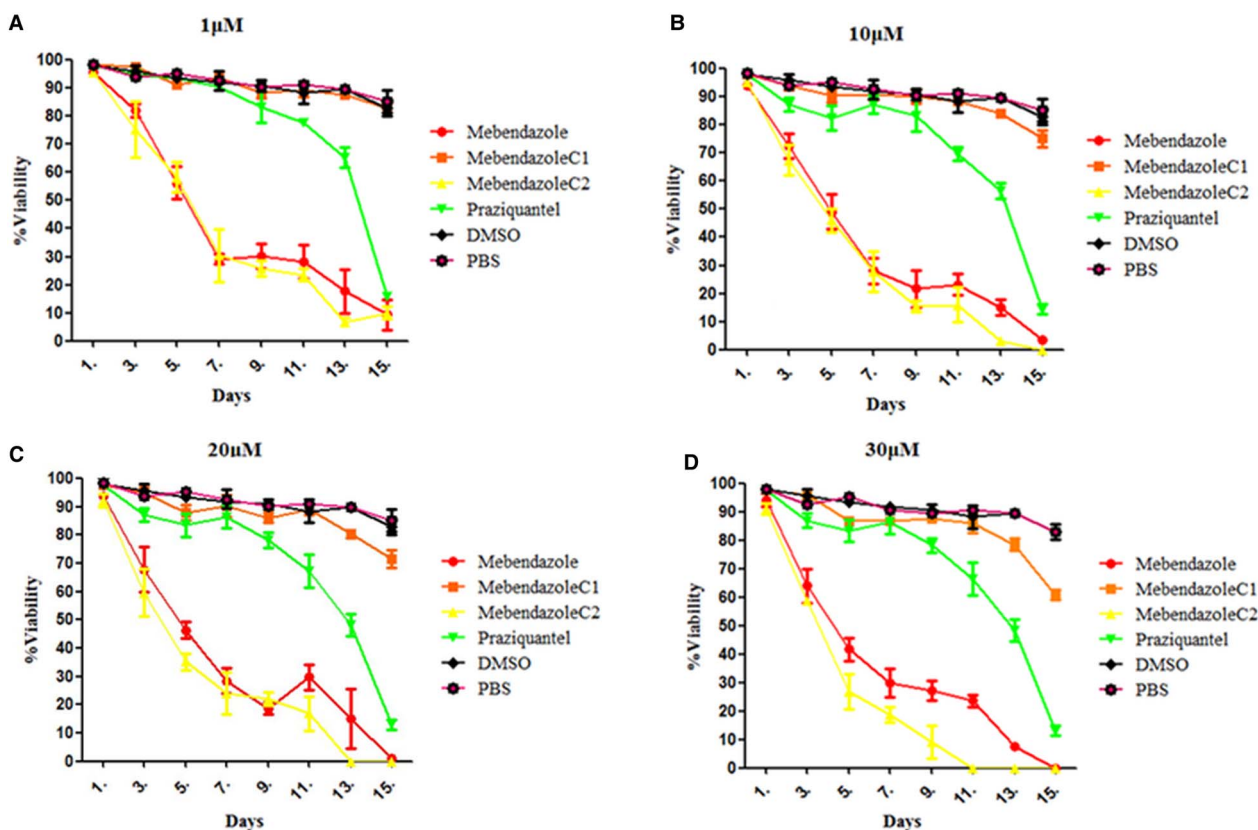


Fig. 3. (Colour online) Effects of compounds to protoscolecids following *in vitro* treatment with compounds at different concentrations. (A) 1 μM , (B) 10 μM , (C) 20 μM and (D) 30 μM . Mean values were derived from three biological repeats.

In vitro drug treatment of *E. multilocularis* protoscolecids

Treatments were performed in 24-well plates containing 2 mL of 1066 medium and 2000 protoscolecids were added to each well. M-C1 and M-C2 were added to each plate separately at final concentrations of 1, 10, 20 and 30 μM . Praziquantel and mebendazole were used as experimental controls, whereas 10% DMSO was used as a negative control. Protoscolecids were incubated at 37 °C, 5% CO₂ without medium changes for a period of 15 days. About 100 protoscolecids (in 100 μL medium) were taken from each well and the mortality of protoscolecids was assessed using Trypan blue stain and visualized on an inverted microscope (Yuan *et al.*, 2016).

In vitro drug treatment of *E. multilocularis* metacystodes

Echinococcus multilocularis metacystodes were collected after 1–2 months in culture and were washed three times in PBS. Drug treatments were performed as described by Stadelmann *et al.* (Küster *et al.*, 2011; Hemer and Brehm, 2012; Stadelmann *et al.*, 2014). Briefly, 1 mL of medium without phenol red (DMEM, 100 U mL⁻¹ penicillin G, 100 μg mL⁻¹ streptomycin sulphate and 2 mM L-glutamine) was added to the same volume of metacystodes and distributed to 24-well plates (25–35 vesicles well⁻¹). The compounds were prepared as stocks of 100 mM in DMSO. Firstly, compounds were diluted with DMSO to a concentration of 1 mM and were then added to the culture medium with final concentrations of 1, 10, 20 and 30 μM . DMSO was used as a negative control. As a positive control, MMV (Medicines Malaria Venture) was applied. The effects of different compounds (1, 10, 20 and 30 μM) on *E. multilocularis* metacystodes after 5

days were assessed by visual damage of metacystodes, which were classified into the following four levels: 0 (<10%), 1 (10–50%), 2 (50–90%) and 3 (>90%).

Morphological investigation of mebendazole C2-treated metacystodes

Echinococcus multilocularis metacystodes incubated with 30 μM M-C2 were collected, washed once with PBS and fixed in 2.5% glutaraldehyde in 100 mM sodium cacodylate buffer (pH 7.2) for 2 h. This was followed by post-fixation in 2% OsO₄ in 100 mM sodium cacodylate buffer for 2 h. Subsequently, specimens were washed in distilled water, treated with 1% uranyl acetate for 30 min, washed again in water and dehydrated by sequential incubations in ethanol (30–50–70–90–100%). For SEM (Scanning Electron Microscopy) analysis, dehydrated specimens were finally immersed in hexamethyl-disilazane and dried under a fume hood. They were then sputter-coated with gold and inspected with a scanning electron microscope operating at 25 kV (Stadelmann *et al.*, 2011, 2016).

Cytotoxicity assay

The *in vitro* cytotoxicity of M-C1 and M-C2 on rat hepatoma (RH) cell lines, after 72 h incubation at different concentrations (1, 10, 20 and 30 μM , respectively), was investigated in comparison with mebendazole under identical conditions with the CCK-8 (Cell Counting Kit-8) assay. RH cells were seeded onto a 96-well microtitre plate at a density of 5000 cells well⁻¹. Cells were cultured in DMEM without phenol red (supplemented with 1% L-glutamine, 10% FCS, 50 U mL⁻¹ penicillin G and

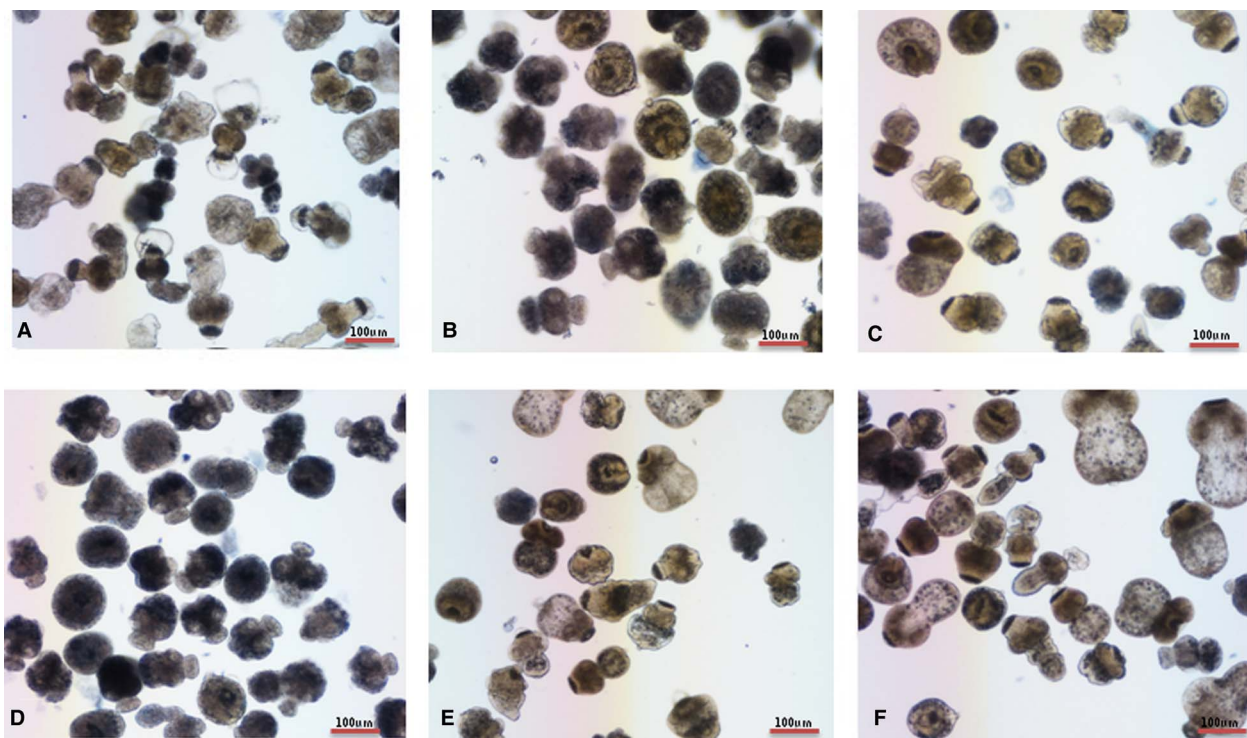


Fig. 4. (Colour online) *In vitro* activities of compounds on *E. multilocularis* protoscoleces. The effects of compounds on protoscoleces were assessed by Trypan blue every each day. Protoscoleces of *E. multilocularis* were exposed to (A) praziquantel, (B) mebendazole, (C) M-C1, (D) M-C2, (E) DMSO and (F) PBS 11 days post-treatment at a concentration of 30 μM .

Table 1. *In vitro* activity of compounds on *E. multilocularis* metacystodes

Compound	Concentrations/damage classification			
	1 μM	10 μM	20 μM	30 μM
Mebendazole	2	2	2	2
Mebendazole C1	1	1	1	1
Mebendazole C2	2	2	2	2
MMV	1	1	2	3
DMSO	1	0	0	1
PBS	1	NA ^a	NA	NA

^aNA, not available.

Effects on *E. multilocularis* metacystodes incubated with compounds (1, 10, 20 and 30 μM , respectively) for 5 days were assessed by visual damaged percentage of metacystodes (0, <10%; 1, 10–50%; 2, 50–90%; 3, >90%).

50 g mL⁻¹ streptomycin) for 72 h at 37 °C, 5% CO₂. Then 10 μL of CCK-8 solution was added to each well and incubated for 1 h in the incubator. The absorbance was measured at 450 nm using a microplate reader (Küster *et al.*, 2013).

Results

Solubility improved by introduction of epoxy group

When dissolved in DMSO-PBS (1:9) solution, mebendazole formed an opaque liquid, with an emulsion floating ('+----'), while M-C1 and M-C2 were relatively clear solutions with only a few tiny particles ('+++--' and '++++-', respectively). MMV completely dissolved without any visible particles or droplets ('+++++') (Fig. 2).

Effects of mebendazole compounds on protoscoleces in vitro

Trypan blue stain indicated that the mortality of protoscoleces was about 70–80% on day 7 post-exposure to mebendazole or M-C2, and M-C2 showed higher parasitocidal effects than mebendazole ($P > 0.05$). The parasitocidal activity of both mebendazole and M-C2 were higher than praziquantel at corresponding concentrations and time points (Fig. 3). The parasitocidal effect of M-C1 was low, and even at a concentration of 30 μM , the mortality of protoscoleces was not more than 40%. DMSO and PBS exhibited no significant effects on protoscoleces. Significant morphological changes (disruption) of protoscoleces were observed on day 11 post-treatment with praziquantel, mebendazole and M-C2 (Fig. 4) compared with DMSO and PBS control groups.

Effects of mebendazole compounds on metacystodes in vitro

In vitro effects of mebendazole compounds on *E. multilocularis* metacystodes were assessed (Table 1). The results demonstrate that the percentage of damaged metacystodes treated with mebendazole and M-C2 at different concentrations is similar. M-C1, DMSO and PBS had almost no effects on metacystodes. The basic trend of the effects of used compounds on metacystodes was similar to that on protoscoleces.

Morphological analysis with light microscopy revealed that in the DMSO-treated group, no observable collapse of the germinal layer from the laminated layer was observed, while in M-C2- and MMV-treated groups, germinal layers detached from laminated layers and germinal cells accumulated in the centre of metacystodes and formed a circular rim (Fig. 5).

Morphological study under SEM revealed that metacystodes treated with PBS and DMSO showed intact/ slight damage in the germinal layer. Ultrastructural changes in metacystodes

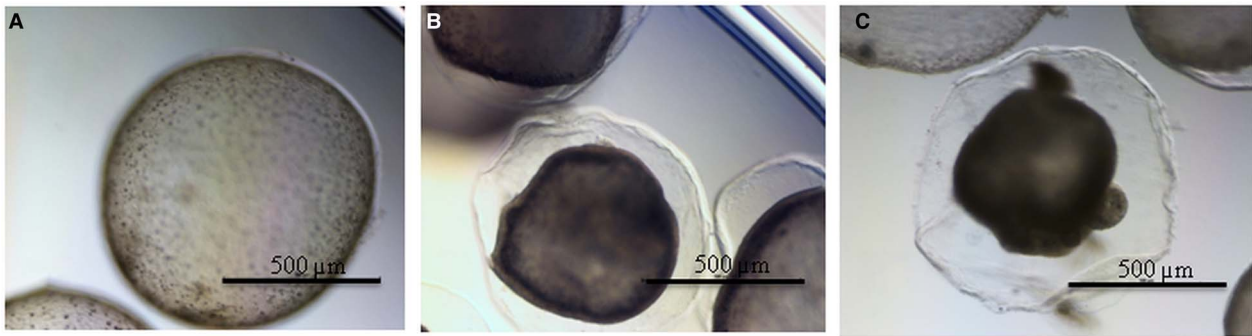


Fig. 5. (Colour online) Morphological changes of *E. multilocularis* metacystodes treated with compounds. (A) DMSO-treated metacystodes, (B) M-C2-treated metacystodes and (C) MMV-treated metacystodes.

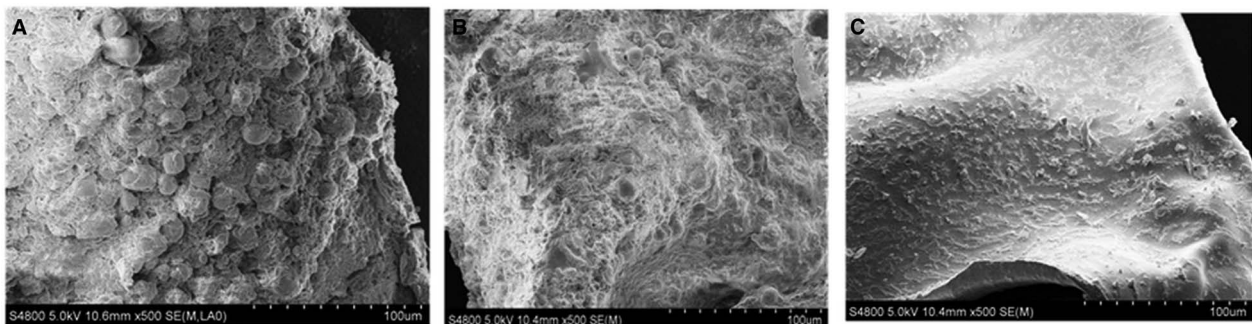


Fig. 6. Ultrastructural changes of *E. multilocularis* metacystodes treated/untreated with M-C2. (A) PBS-treated metacystodes, (B) DMSO-treated metacystodes and (C) M-C2-treated metacystodes.

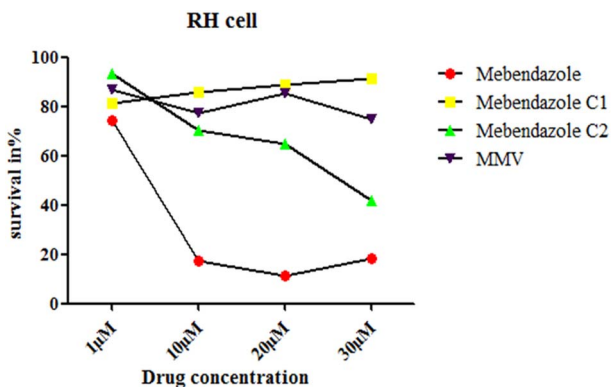


Fig. 7. (Colour online) Survival rate of RH cells incubated in different compounds at different concentrations. The cytotoxicity to RH cell was examined by CCK-8 assay. The term 'survival in %' in Fig. 7 relates to the fluorescence measured at 450 nm.

treated with M-C2 were significant—overall damage of germinal cells and thus the collapse of the germinal layer (Fig. 6).

Cytotoxicity of different compounds in RH cells

The introduction of the epoxy group not only improved the solubility of mebendazole but also reduced the cytotoxicity of mebendazole in RH cells. The cytotoxicity of M-C1 was lower than M-C2, and MMV exhibited the lowest toxicity (Fig. 7).

Discussion

Benzimidazoles are a class of compounds with a broad biological activity and are widely applied in medical fields to treat cancer (Abdel-Mohsen *et al.*, 2010) as well as bacterial (Patel *et al.*,

2012), viral (Luo *et al.*, 2010) and parasitic infections (Meng *et al.*, 2009; Hernández-Luis *et al.*, 2010; Saify *et al.*, 2012; Li and Zhang, 2013). To date, mebendazole and albendazole are the main drugs used to treat echinococcosis (Teggi *et al.*, 1993). However, their poor intestinal absorption results in low parasitocidal effects and thus there is an urgent need to develop new formulations that will increase bioavailability and therapeutic efficacy (Liu and Zhang, 2009).

A previous study demonstrated that the introduction of phosphonoxymethyl to albendazole N1 resulted in both high solubility and stability in water (Chassaing *et al.*, 2008). In our present study, the introduction of an epoxy group to mebendazole N1 or N3 also greatly improved its solubility (Fig. 2). These studies suggest that the introduction of certain chemical groups to certain sites of mebendazole may change its hydrophilicity and thus improve its solubility.


Both M-C1 and M-C2 exhibited higher solubility than mebendazole, while only M-C2 exhibited higher parasitocidal activity on protoscolexes than mebendazole 7 days post-treatment (Figs 3 and 4). Mebendazole and M-C2 are more effective ($P < 0.05$) than praziquantel (which was used as a positive control) at corresponding concentrations and time points (Fig. 3). Although the solubility of M-C1 is better than that of M-C2, its parasitocidal effect is not as good as M-C2. The number of damaged metacystodes in the M-C2-treated group was greater than in the M-C1-treated group (Table 1). These results indicate that the improvement of drug solubility by the introduction of a chemical group to mebendazole will not necessarily increase the parasitocidal effect, and the impact on its effective conformation is also one of the most important factors that should be considered. Further studies are needed to elucidate how the introduction of an epoxy group affects the conformation of mebendazole and thus its efficacy.

Significant ultrastructural changes were observed in metacystodes treated with M-C2, namely an overall damage of germinal cells and thus collapse of the germinal layer. This is because the compounds penetrated the external protective laminated layer of metacystodes, which protects the parasite against host attack and immune responses (Díaz *et al.*, 2011, 2016; Díaz, 2017). Such drug penetration is fatal, as it directly affects the normal development and metabolism of germinal cells on the laminated layer.

The introduction of an epoxy group to mebendazole not only improved its solubility but also reduced its cytotoxicity in RH cells (Fig. 7). Compared with M-C2, the toxicity of M-C1 was lower. M-C1 kept a non-concentration-dependent trend, while for M-C2, the cell inhibition rate increased from 1 to 30 μM . Further studies are needed to determine the side-effects (including cytotoxicity to different organs) and appropriate concentrations of these compounds *in vivo*.

Conclusions

This study has evaluated the parasitocidal effects of mebendazole derivatives with improved solubility and examined their cytotoxicity in RH cells. The new derivative M-C2 exhibits both improved solubility and strong parasitocidal activity against *E. multilocularis* protoscoleces and metacystodes *in vitro*. Additionally, M-C2 exhibited lower cytotoxicity in RH cells. Medical applications of mebendazole have been restricted, due to its low solubility and thus low bioavailability in the human body. Therefore, the improvement of mebendazole solubility is one of the key strategies to improve its effectiveness in treating echinococcosis. This study indicates that M-C2 may be a potent drug candidate for further research on echinococcosis treatment.

Author ORCIDs.  Fuqiang Huang, 0000-0002-3613-2350; Yumin Zhao, 0000-0001-5378-0328

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Conflict of interest. None.

Ethical standards. Animal care and all animal procedures were carried out in compliance with the Guidelines for the Care and Use of Laboratory Animals produced and approved by the Ethics Committee of the Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (No. IPD-2016-21).

Consent for publication. Not applicable.

Availability of data and material. The datasets supporting the conclusions of this article are included within the article.

References

- Abdel-Mohsen HT, Ragab FA, Ramla MM and El Diwani HI (2010) Novel benzimidazole-pyrimidine conjugates as potent antitumor agents. *European Journal of Medicinal Chemistry* **45**, 2336–2344.
- Ao Qier, Wen H and Yang WG (2003) Comparative observation on efficacy of liposomal albendazole against infection with *Echinococcus granulosus* by oral and intraperitoneal injection. *Endemic Diseases Bulletin* **18**, 20–24.
- Chai JJ, Menghebat, Jiao W, Sun DY, Liang B, Shi J, Fu C, Li X, Mao Y, Wang X, Dolikun, Guliber, Wang Y, Gao F and Xiao S (2001) Clinical efficacy of albendazole emulsion in treatment of 212 cases of liver cystic hydatidosis. *Chinese Journal of Parasitology and Parasitic Diseases* **19**, 129–133.
- Chassaing C, Berger M, Heckerth A, Ilg T, Jaeger M, Kern C, Schmid K and Uphoff M (2008) Highly water-soluble prodrugs of anthelmintic benzimidazole carbamates: synthesis, pharmacodynamics, and pharmacokinetics. *Journal of Medicinal Chemistry* **51**, 1111–1114.
- Díaz A (2017) Immunology of cystic echinococcosis (hydatid disease). *British Medical Bulletin* **124**, 121–133.
- Díaz A, Casaravilla C, Allen JE, Sim RB and Ferreira AM (2011) Understanding the laminated layer of larval *Echinococcus* II: immunology. *Trends in Parasitology* **27**, 264–273.
- Díaz A, Casaravilla C, Barrios AA and Ferreira AM (2016) Parasite molecules and host responses in cystic echinococcosis. *Parasite Immunology* **38**, 193–205.
- Flores-Ramos M, Ibarra-Velarde F, Hernández-Campos A, Vera-Montenegro Y, Jung-Cook H, Cantó-Alarcón GJ, Del Rivero LM and Castillo R (2014) A highly water soluble benzimidazole derivative useful for the treatment of fasciolosis. *Bioorganic & Medicinal Chemistry Letters* **24**, 5814–5817.
- Hemer S and Brehm K (2012) *In vitro* efficacy of the anticancer drug imatinib on *Echinococcus multilocularis* larvae. *International Journal of Antimicrobial Agents* **40**, 458–462.
- Hernández-Luis F, Hernández-Campos A, Castillo R, Navarrete-Vázquez G, Soria-Arteche O, Hernández-Hernández M and Yépez-Mulia L (2010) Synthesis and biological activity of 2-(trifluoromethyl)-1H-benzimidazole derivatives against some protozoa and *Trichinella spiralis*. *European Journal of Medicinal Chemistry* **45**, 3135–3141.
- Jasmer DP, Yao C, Rehman A and Johnson S (2000) Multiple lethal effects induced by a benzimidazole anthelmintic in the anterior intestine of the nematode *Haemonchus contortus*. *Molecular and Biochemical Parasitology* **105**, 81–90.
- Jiang CP (2002) Today's regional distribution of echinococcosis in China. *Chinese Medical Journal* **115**, 1244–1247.
- Küster T, Stadelmann B, Hermann C, Scholl S, Keiser J and Hemphill A (2011) *In vitro* and *in vivo* efficacies of mefloquine-based treatment against alveolar echinococcosis. *Antimicrobial Agents and Chemotherapy* **55**, 713–721.
- Küster T, Kriegl N, Boykin DW, Stephens CE and Hemphill A (2013) *In vitro* and *in vivo* activities of dicationic diguanidino compounds against *Echinococcus multilocularis* metacystodes. *Antimicrobial Agents and Chemotherapy* **57**, 3829–3835.
- Li YJ and Zhang JJ (2013) Advances in the study of biologic activity of benzimidazole compounds. *Chemical Engineering & Equipment* **8**, 175–177.
- Li HT, Wen H and Zhao JM (2010) Current status of clinical treatment of human hydatid disease. *Chinese Journal of Surgery* **08**, 711–713.
- Liu CS and Zhang HB (2009) Research progress on the efficacy, metabolism and bioavailability of mebendazole in hydatid disease. *Chinese Journal of Parasitology Parasitic Diseases* **27**, 513–519.
- Liu C, Zhang H, Yin J and Hu W (2015) *In vivo* and *in vitro* efficacies of mebendazole, mefloquine and nitazoxanide against cyst echinococcosis. *Parasitology Research* **114**, 2213–2222.
- Luo Y, Yao JP, Yang L, Feng CL, Tang W, Wang GF, Zuo JP and Lu W (2010) Design and synthesis of novel benzimidazole derivatives as inhibitors of hepatitis B virus. *Bioorganic & Medicinal Chemistry* **18**, 5048–5055.
- Meng JP, Geng RX, Zhou CH and Gan LL (2009) Advances in the research of benzimidazole drugs. *Chinese Journal of New Drugs* **18**, 1505–1514.
- Mottier L, Alvarez L, Ceballos L and Lanusse C (2006) Drug transport mechanisms in helminth parasites: passive diffusion of benzimidazole anthelmintics. *Experimental Parasitology* **113**, 49–57.
- Patel RV, Patel PK, Kumari P, Rajani DP and Chikhaliya KH (2012) Synthesis of benzimidazolyl-1,3,4-oxadiazol-2ylthio-N-phenyl (benzothiazolyl) acetamides as antibacterial, antifungal and antituberculosis agents. *European Journal of Medicinal Chemistry* **53**, 41–51.
- Saify ZS, Azim MK, Ahmad W, Nisa M, Goldberg DE, Hussain SA, Akhtar S, Akram A, Arayne A, Oksman A and Khan IA (2012) New benzimidazole derivatives as antiplasmodial agents and plasmepsin inhibitors: synthesis and analysis of structure-activity relationships. *Bioorganic & Medicinal Chemistry Letters* **22**, 1282–1286.
- Spiliotis M and Brehm K (2009) Axenic *in vitro* cultivation of *Echinococcus multilocularis* metacystode vesicles and the generation of primary cell cultures. *Methods in Molecular Biology* **470**, 245–262.

- Stadelmann B, Küster T, Scholl S, Barna F, Kropf C, Keiser J, Boykin DW, Stephens CE and Hemphill A** (2011) *In vitro* efficacy of dicationic compounds and mefloquine enantiomers against *Echinococcus multilocularis* metacestodes. *Antimicrobial Agents and Chemotherapy* **55**, 4866–4872.
- Stadelmann B, Aeschbacher D, Huber C, Spiliotis M, Müller J and Hemphill A** (2014) Profound activity of the anti-cancer drug bortezomib against *Echinococcus multilocularis* metacestodes identifies the proteasome as a novel drug target for cestodes. *PLoS Neglected Tropical Diseases* **8**, e3352.
- Stadelmann B, Rufener R, Aeschbacher D, Spiliotis M, Gottstein B and Hemphill A** (2016) Screening of the open source malaria box reveals an early lead compound for the treatment of alveolar echinococcosis. *PLoS Neglected Tropical Diseases* **10**, e0004535.
- Teggi A, Lastilla MG and De Rosa F** (1993) Therapy of human hydatid disease with mebendazole and albendazole. *Antimicrobial Agents and Chemotherapy* **37**, 1679–1689.
- Yuan M, Luo Y, Xin Q, Gao H, Zhang G and Jing T** (2016) Efficacy of osthole for *Echinococcus granulosus* *in vitro* and *Echinococcus multilocularis* *in vivo*. *Veterinary Parasitology* **226**, 38–43.