Acta Tropica 185 (2018) 138–143

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Short Communication

In vitro and *in vivo* efficacies of carbazole aminoalcohols in the treatment of alveolar echinococcosis

Zhisheng Dang[a,1], Shuo Xu[b,1], Haobing Zhang[a], Weifeng Gui[b], Yumin Zhao[b], Liping Duan[a,*], Wei Hu[a,*]

[a] National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Key Laboratory of Parasite and Vector Biology, MOH, National Center for International Research on Tropical Diseases, WHO Collaborating Centre for Tropical Diseases, Shanghai 200025, PR China
[b] Department of Parasitology, School of Basic Medicine, Guilin Medical University, Guilin, Guangxi 541004, PR China

ARTICLE INFO

Keywords:
Echinococcus multilocularis
Carbazole aminoalcohols
Effect
Toxicity

ABSTRACT

Benzimidazoles, including albendazole and mebendazole, are the major drugs for clinical chemotherapy of echinococcosis. They mainly exert parasitostatic effects depending on high dosages for long-term. Previous studies have identified carbazole aminoalcohols as novel anti-CE (cystic echinococcosis) agents. However, it is still to be confirmed whether it is effective on alveolar echinococcosis (AE) or not. In the present study, efficacies of novel carbazole aminoalcohols, propylamine, R-propylamine and S-propylamine were evaluated under *in vitro* and in *vivo* conditions. Carbazole aminoalcohols were tested against *Echinococcus multilocularis* (*E. multilocularis*) protoscoleces (PSC) *in vitro*. The effects of propylamine and R-propylamine exhibited a time-dependent manner at different concentrations, while the effect of S-propylamine was very poor. At a concentration of 20 μM, the mortality of PSC achieved to 100% on the 11th day after exposure to R-propylamine. The treatment of carbazole aminoalcohols to infected mice resulted in statistically significant reductions in the cyst weights compared with those obtained from negative control mice ($p < 0.05$), and no significant differences were found between albendazole and carbazole aminoalcohols ($p > 0.05$). The cytotoxicity examination in rat hepatoma (RH) cells indicated that propylamine and R/S-propylamine were lower that of albendazole at a low concentration (5 μM). In addition, histopathological observation of organs (liver, spleen and kidney) for experimental mice showed mild inflammatory changes in the liver and spleen. This study reveals the potential of carbazole aminoalcohols as a class of novel anti-AE agents.

1. Introduction

Alveolar echinococcosis (AE), also known as alveolar hydatid disease, is a zoonosis caused by infection with the larva of *E. multilocularis* in visceral organs of the human beings and ruminant animals. The disease is endemic mainly in Northern Hemisphere including the northwest of China, a region where the livestock industry is the main economic activity (Mihmanli et al., 2016). Nomadic and semi-nomadic lifestyles constitute the most important risk factor for hydatid disease in this region (Hemphill et al., 2014). Normally, 70% of the hydatid disease cases report liver infection. However, other organs, such as the lung, brain, kidney, heart, bone marrow cavity and orbit can be infected. The main pathological effect of this disease is a series of complications inflicted on the human body and hypotrophy of various organs (Kuster et al., 2014; Liu et al., 2015). Clinical manifestation of *E. multilocularis* is similar to that of liver cancer. The degree of malignancy is high, and the pathogenicity is strong. Disability and fatality rates are also higher. It has also been associated with permanent, adverse health effects in humans (Pakala et al., 2016).

The current strategies for treating human AE are surgical resection of the parasite mass complemented by chemotherapy with benzimidazoles, such as albendazole or mebendazole and, for inoperable cases, chemotherapy alone is applied (Teggi et al., 1993; Hemphill et al., 2014). Albendazole treatment has been proven to inhibit parasite proliferation but is rarely curative, resulting in a long-term of treatment, high costs and an elevated risk of adverse effects (Aasen et al., 2016).

If the disease is detected early, then immediate implementation of surgical resection combined with albendazole treatment can lead to effective cure. However, the diagnosis of this disease is often in the late stage. At the late stage, it is very difficult to remove the lesion completely, even through surgical resection. Moreover, the disease easily relapses when treatment is implemented in late stage. This situation

* Corresponding authors.
E-mail addresses: duanlp@nipd.chinacdc.cn (L. Duan), huwei@nipd.chinacdc.cn (W. Hu).
[1] Joint first author.

https://doi.org/10.1016/j.actatropica.2018.05.007
Received 2 March 2018; Received in revised form 3 May 2018; Accepted 6 May 2018
Available online 07 May 2018
0001-706X/ © 2018 Elsevier B.V. All rights reserved.

deems it urgent to develop more effective drugs for prevention and treatment in order to guarantee the safety of the lives of a large population of farmers and herdsmen living in endemic areas. Effective treatment programmes are likely to promote the healthy development of animal husbandry economy.

Carbazole is aprominentcore structure found in numerous natural and synthetic compounds with wide range of biological activities, including antineoplastic and neuroprotective effects (Wang et al., 2016). Carbazole aminoalcohols has also been demonstrated as having broad spectrum anti-parasite effects on *Plasmodium falciparum* and *Schistosoma japonicum* (Wang et al., 2017b). Some of carbazole derivatives exhibit antitumor activity by targeting DNA topoisomerases (Saulnier et al., 2005; Nakamura et al., 2003). Sequence analyses showed that *E. granulosus* topoisomerase I (*Eg*topo I) shares 55.8% identity with human topo I, inferring that carbazole aminoalcohols in humans is also a drug target for *E. granulosus* (Wang et al., 2017b). In previous study (Wang et al., 2017a), a series of carbazole aminoalcohol derivatives were designed and synthesized and a phenotypic *in vivo* screening against cystic echinococcosis was performed for carbazole aminoalcohols. Notably, among the test compounds, *in vivo* efficacy of compound 2 (propylamine) (see Fig. 1) against CE showed a highly significant reduction in parasite burden in comparison with mice treated with albendazole. Thus, it is believed that carbazole aminoalcohols are lead compounds against AE.

In the present study, we evaluated the *in vitro* parasiticidal efficacy of synthetic carbazole aminoalcohols (propylamine and its chiral

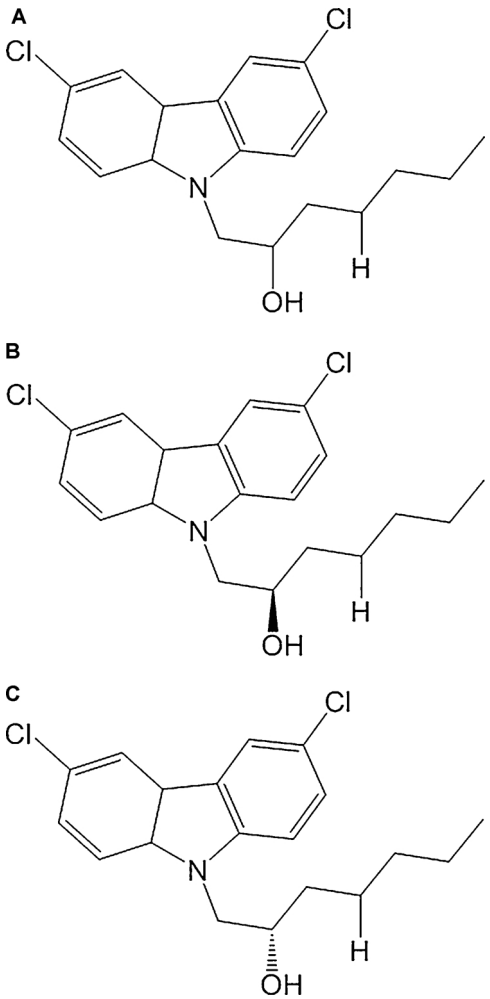

Fig. 1. Structure of propylamine (A), R-propyliamine (B) and S-propylamine (C).

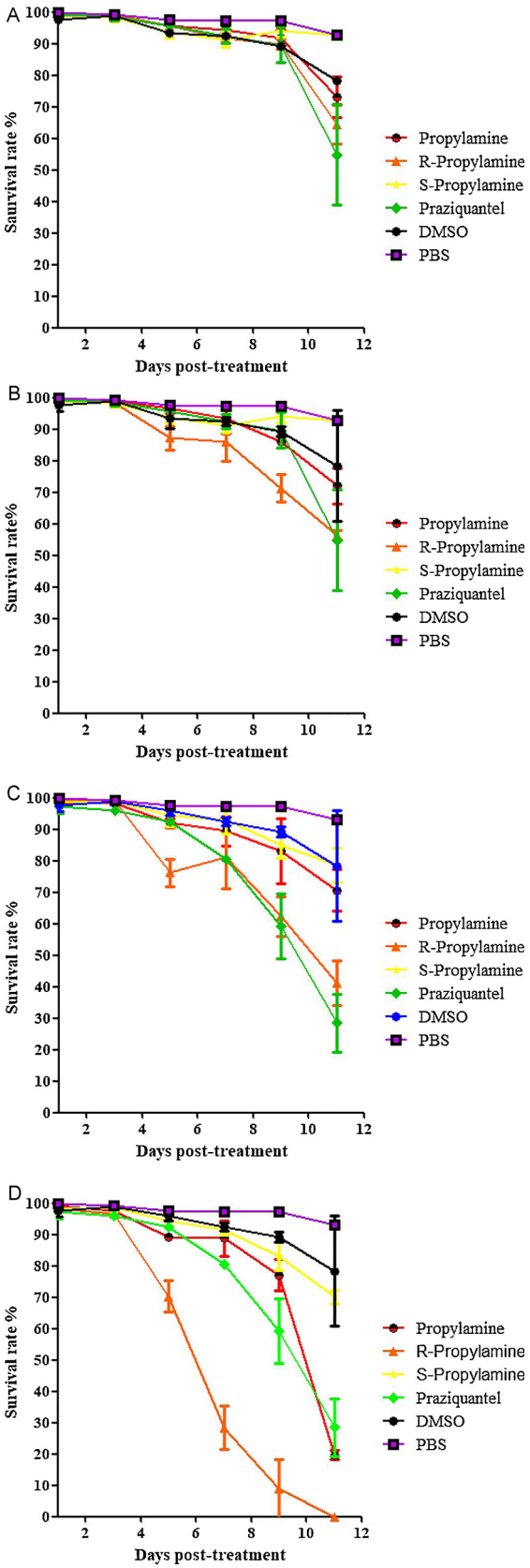

Fig. 2. Parasiticidal activity of experimentally used compounds against PSCs. *E. multilocularis* PSCs were cultured for 11 days *in vitro* in the presence of propylamine, R/S-propylamin and praziquantel as a positive control at (A) 5 μM, (B) 10 μM, (C) 15 μM and (D) 20 μM.

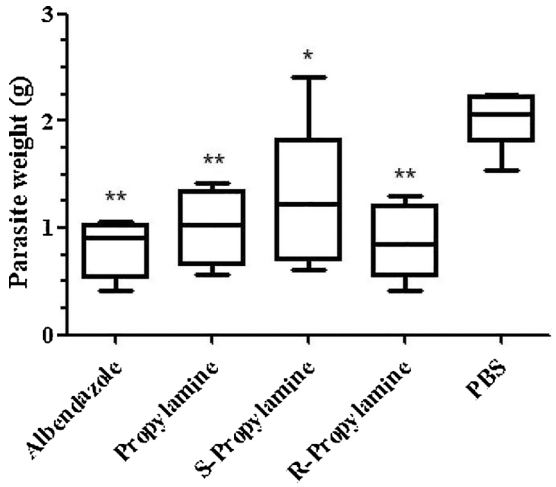

Fig. 3. *In vivo* evaluation of compounds for treatment of *E. multilocularis*-infected mice.
After necropsy of *in vivo* treated mice, the average parasite weight in each group (n = 4, 6, 7, 5, 6 separately) was calculated and statistically analyzed.
** $p < 0.01$; * $p < 0.05$

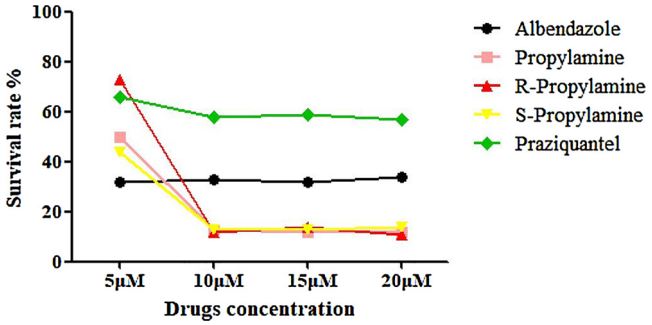

Fig. 4. Cytotoxicity to RH cells was measured by CCK-8 assay at different concentrations (5, 10, 15 and 20 μM) of used compounds.

isomers S-propylamine and R-propylamine) against *E. multilocularis* PSCs. In addition, *in vivo* chemotherapeutic effects of the compounds were also assessed using experimentally infected mouse model.

2. Materials and methods

2.1. Materials and reagents

The synthesis of carbazole aminoalcohols is described in the supplementary data available at JAC Online (Wang et al., 2017a). Unless otherwise specified, all chemicals were purchased from Sigma-Aldrich.

2.2. Structure of compounds

A Propylamine
B R-propylamine
C S-propylamine

2.3. Parasites, animals and infection

Four-weeks-aged male mice (BALB/c strain) were purchased from SLAC Laboratory Animal Center (Shanghai, China). A mouse successfully infected with *E. multilocularis* (Qinghai strain) was sacrificed and homogenized metacestode materials were collected. The PSCs were purified for determination of survival rate. The experimental mice were inoculated with homogenized metacestode material containing 2000 PSCs intraperitoneally (i.p.) for a period of one month.

2.4. Ethics statement

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 National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (No.IPD-2016-21).

2.5. In vitro culture of E. multilocularis PSCs and drug screening

Aseptic manipulation was performed in a mouse (BALB/c strain) alveolar echinococcosis model. The secondary alveolar hydatid tissue of mice was cut and then washed with phosphate-buffered saline (PBS). Afterwards, the tissue was precipitated naturally within 20 mins, and then washed 3 times with PBS. The PSCs were then collected and activity examined by trypan blue staining. In subsequent experiments, a survival rate of more than 95% is required (Kuster et al., 2011; Spiliotis and Brehm, 2009). Treatments were performed using 24-well tissue culture plates containing 2000 PSCs (in 2 mL of 1066 medium) per well. The medium was supplemented with propylamine, R/S-propylamine at final concentrations of 5, 10, 15, and 20 μM. Praziquantel were used as positive control, whereas 10% DMSO and PBS were used as negative controls. The mortality of PSCs was assessed using a trypan blue staining and visualized on an inverted microscope at 40× magnification (Kuster et al., 2013).

2.6. Experimental infection of mice and in vivo treatment

Forty 4-weeks-aged female BALB/c mice (20–25 g) were divided into 5 groups (8 animals per group). 0.2 mL of homogenized metacestode material suspension containing 2000 live PSCs was inoculated to mice intraperitoneally (i.p.) (Ceballos et al., 2009; Kuster et al., 2011; Nicolao et al., 2014; Spicher et al., 2008). At 4 weeks post-infection, treatments were carried out as follows. All compounds were applied on a daily basis at a dose of 200 mg/kg of body weight. All compounds were performed by suspending the drugs in a 1:1 mixture of honey and 0.5% carboxymethyl cellulose sodium salt (CMC) and feeding the appropriate amount to the animals. The negative control group was administered with PBS. Mice were administered with prepared solutions everyday (Ceballos et al., 2011; Pensel et al., 2015). After 6 weeks post-infection, the mice were euthanized. Necroscopy was performed and the cysts were collected from the peritoneal cavity and liver, in order to determine parasite weight.

2.7. Cytotoxicity assay

The cytotoxicity of all compounds to RH cell was investigated by CCK-8 assay after 72 h incubation under different concentrations. Cells were seeded in a 96-well microtiter plate (Greiner Bio-One) at a density of 5000 cells/well. They were cultured at 37 °C, 5% CO2 in DMEM without phenol red (supplemented with 1% L-glutamine, 10% FCS, 50 U/ml penicillin G, and 50 g/ml streptomycin) for 24 h. 10 μL of the CCK-8 solution was added to each well and incubated for 1 h. The absorbance was then measured at 450 nm using a microplate reader (Stadelmann et al., 2011).

2.8. Visceral histopathological observation of experimental animals

At 6 weeks post-treatment, the mice were euthanized. The liver, spleen and kidney of mice were collected. Slices were prepared and stained by Hematoxylin and Eosin (HE) for histopathological observation under microscope.

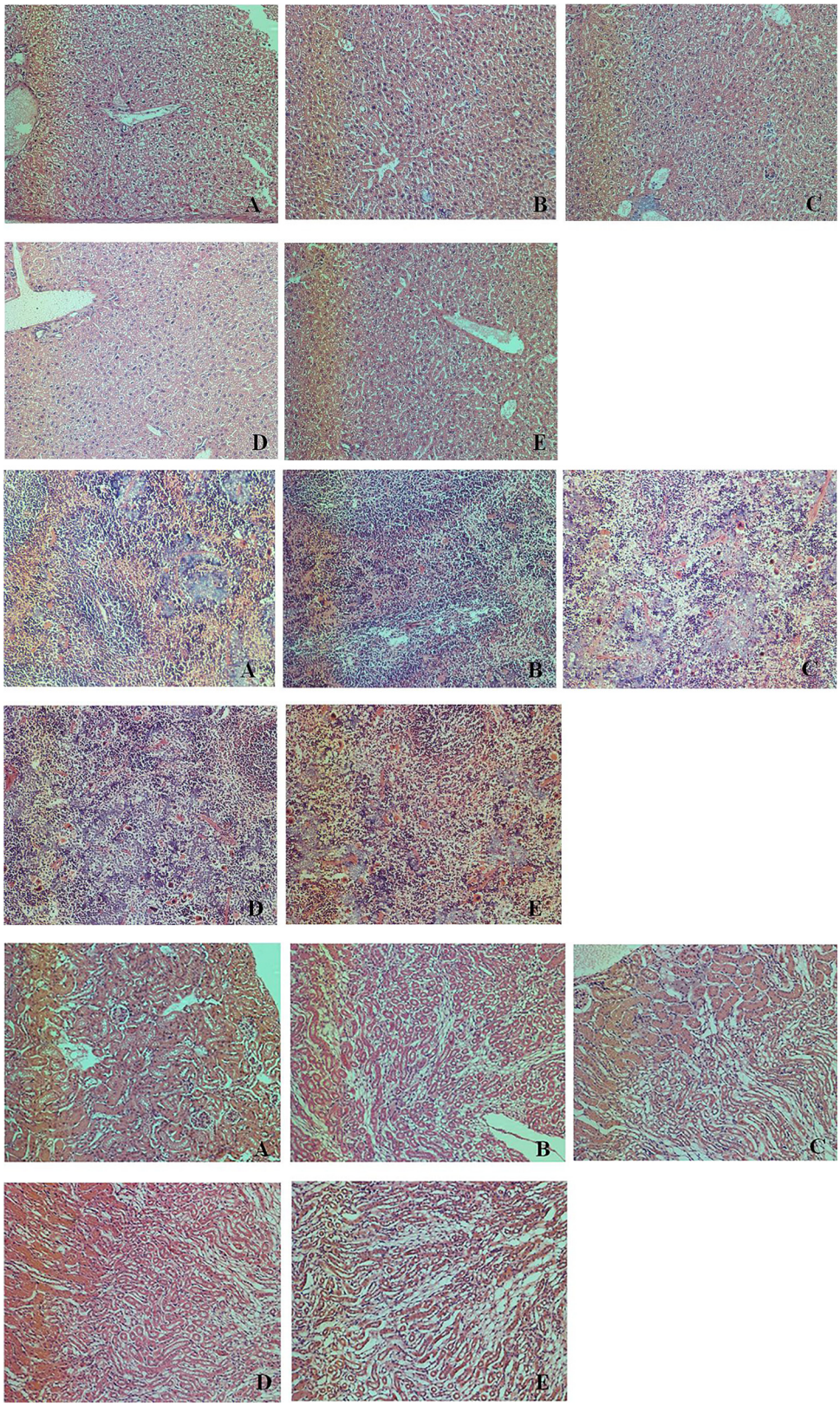

Fig. 5. Visceral histopathological observation.
Slices of the liver, spleen and kidney were prepared and stained by Hematoxylin and Eosin (HE, 200×) and observed under microscope. For each organ, (A) Albendazole group; (B) Propylamine group; (C) R-propylamine group; (D) S-propylamine and (E) PBS group.

3. Results

3.1. Carbazole aminoalcohols exhibit potent parasiticidal activity against E. multilocularis PSC in vitro

After trypan blue stain of compounds-treated PSCs, the mortality was calculated and the parasiticidal effects were evaluated. Overall, propylamine, R-propylamine and praziquantel had significant effects on *E. multilocularis* larval stage (PSC) *in vitro* in a time-dependent manner at different concentrations (Fig. 2). Especially, on day 11 post-treatment, PSC mortality reached > 30% when treated by praziquantel and R-propylamine at 5 μM and 10 μM (Fig. 2A, B), which increased dramatically to about 60% and 70% respectively, at 15 μM (Fig. 2C); notably, at 20 μM (Fig. 2D), R-propylamine exhibited the highest parasiticidal activity (a mortality of 100% for PSCs). Meanwhile, parasiticidal effects of S-propylamine was not over 30% at 5, 10 and 15 μM, but increased dramatically to 70% at 20 μM. The killing effect of S-propylamine to PSC was poor, and even at a concentration of 20 μM, the mortality of PSC was not more than 30%.

3.2. Significant activity of R-propylamine in treatment of infected mice

The *in vivo* efficacy of all compounds against *E. multilocularis* was investigated in BALB/c mice secondly infected with PSCs. After necropsy of mice at 6 weeks post-infection, the average parasite weight in each mouse was calculated (Fig. 3). The results showed that, compared with PBS group, the treatment by albendazole, propylamine and R-propylamine resulted in a significant cysts weight reduction ($p < 0.01$), while S-propylamine showed less cysts weight reduction ($p < 0.05$). There had no significant statistical difference between albendazole, propylamine, R-propylamine and S-propylamin ($p > 0.05$).

3.3. Cytotoxicity of different compounds to RH cells

CCK-8 assay demonstrated that, at the relative high concentrations (10–20 μM), the toxicity of propylamine and R/S-propylamine was higher than albendazole (Fig. 4), while at the low concentration (5 μM), their toxicity was lower than that of albendazole. Praziquantel was observed to have the lowest toxicity regardless of concentration.

3.4. The histopathological observation of experimental mice

For liver from all groups, liver blood sinuses were slightly broadened. Scattered infiltration of hepatic sinusoids was found in all groups except albendazole group. The liver is oedematous and chronically inflamed in albendazole group (Fig. 5A).

For spleen from the albendazole and propylamine groups, the red pulp had expanded a little and the megakaryocyte were scattered. Other groups showed no abnormality (Fig. 5B).

For kidney from all groups, the observation showed no abnormality (Fig. 5C).

4. Discussion

Benzimidazoles are widely applied in the medical field, such as anticancer, antibacterial, antiviral and anti-parasitic diseases (Farahat et al., 2018). To date, mebendazole and albendazole are the main choice for echinococcosis treatment (Filippou et al., 2007; Teggi et al., 1993). However, due to their poor intestinal absorption and parasiticidal effects (Liu and Zhang, 2009), it is urgently needed to develop new drugs to improve its therapeutic efficacy.

From our results, it is clearly seen that propylamine and R-propylamine had significant effects on *E. multilocularis* larval stage (PSC) *in vitro* in a time-dependent manner at different concentrations, similar to the effects to *E. granulosus* reported by (Wang et al., 2017a), while the effect of S-propylamine was very poor (Fig. 2). At a concentration of 20 μM, R-propylamine was significantly effective on PSCs, from the 5th day post-treatment than others including praziquantel (the positive control). *In vivo* study also indicated that, similar to albendazole, R-propylamin produced a very significant cyst reduction in infected mice compared with PBS control ($p < 0.01$) (Fig. 3). In both *in vitro* and *In vivo* experiments, S-propylamine was less effective against *E. multilocularis*, which is quite different with the results from previous studies on *E. granulosus* (Wang et al., 2017a). Whether there is a species-specific effect on *Echinococcus* associated with the variation of drug targets, the further work is needed to identify the exact drug targets for propylamine in order to reveal their mechanisms on *E. multilocularis* and *E. granulosus*.

Although *in vitro* study showed strong activity of propylamin and R-propylamin on PSC (Fig. 2), the cytotoxicity assay showed a relatively higher toxicity to RH cells as well (Fig. 4), which is also a problem often encountered in the development of new drugs (Lv, 2006). After the autopsy of infected mice, damages to main organs such as liver, kidney and spleen were investigated by histopathological observation (Fig. 5). Although there were no significant damages observed in the organs of treated mice, mild inflammatory reaction was confirmed in liver and kidney except spleen. Inflammatory reaction might be related to hydatid infection of mice (Beigh et al., 2017; Zheng et al., 2013). Organ damages by medicine usually happen after a long-term high-dose use (Atici et al., 2005). Therefore, long-term low-dose use is also considered as a therapeutic strategy in order to reduce toxic and side effects. Interestingly, we noticed that compared with albendazole, the toxicity of R-propylamine was lower at a low concentration (5 μM) (Fig. 4), while the mortality of PSCs by R-propylamine at the low concentration is close to 40% (Fig. 2A), implying long-term low-dose use of R-propylamine is a potent strategy for the treatment of *Echinococcus* with reduced toxicity. Such speculation will be verified by our future experiments, which arms to explore the potential of propylamine in echinococcosis therapy.

Financial support

This study was financially supported by Technical Reserve Research Fund for Prevention and Control (CB-16001), National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention.

Acknowledgment

We thank Dr. Joseph W. Magona (African Union-Interafrican Bureau for Animal Resources) for his critical reading of this manuscript.

References

Aasen, T.D., Nasrollah, L., Seetharam, A., 2016. Drug-induced liver failure requiring liver transplant: report and review of the role of albendazole in managing echinococcal infection. Exp. Clin. Transplant. http://dx.doi.org/10.6002/ect.2015.0313.

Atici, S., Cinel, I., Cinel, L., Doruk, N., Eskandari, G., Oral, U., 2005. Liver and kidney toxicity in chronic use of opioids: an experimental long term treatment model. J. Biosci. 30 (2), 245–252.

Beigh, A.B., Darzi, M.M., Bashir, S., Kashani, B., Shah, A., Shah, S.A., 2017. Gross and histopathological alterations associated with cystic echinococcosis in small ruminants. J. Parasit. Dis. 41, 1028–1033.

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

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

Farahat, A.A., Ismail, M.A., Kumar, A., Wenzler, T., Brun, R., Paul, A., Wilson, W.D., Boykin, D.W., 2018. Indole and benzimidazole bichalcophenes: synthesis, DNA binding and antiparasitic activity. Eur. J. Med. Chem. 143, 1590–1596.

Filippou, D., Tselepis, D., Filippou, G., Papadopoulos, V., 2007. Advances in liver echinococcosis: diagnosis and treatment. Clin. Gastroenterol. Hepatol. 5 (2), 152–159.

Hemphill, A., Stadelmann, B., Rufener, R., Spiliotis, M., Boubaker, G., Muller, J., Muller, N., Gorgas, D., Gottstein, B., 2014. Treatment of echinococcosis: albendazole and mebendazole–what else? Parasite 21, 70.

Kuster, T., Stadelmann, B., Hermann, C., Scholl, S., Keiser, J., Hemphill, A., 2011. In vitro and in vivo efficacies of mefloquine-based treatment against alveolar echinococcosis. Antimicrob. Agents Chemother. 55, 713–721.

Kuster, T., Kriegel, N., Boykin, D.W., Stephens, C.E., Hemphill, A., 2013. In vitro and in vivo activities of dicationic diguanidino compounds against *Echinococcus multilocularis* metacestodes. Antimicrob. Agents Chemother. 57, 3829–3835.

Kuster, T., Stadelmann, B., Aeschbacher, D., Hemphill, A., 2014. Activities of fenbendazole in comparison with albendazole against *Echinococcus multilocularis* metacestodes in vitro and in a murine infection model. Int. J. Antimicrob. Agents 43, 335–342.

Liu, C.S., Zhang, H.B., 2009. Research progress on the efficacy, metabolism and bioavailability of mebendazole in hydatid disease. Chin. J. Parasitol. Parasit. Dis. 27 (6), 513–519.

Liu, C., Zhang, H., Yin, J., Hu, W., 2015. *In vivo* and *in vitro* efficacies of mebendazole, mefloquine and nitazoxanide against cyst echinococcosis. Parasitol. Res. 114, 2213–2222.

Lv, Q.J., 2006. Methodology of tocicological exploitation in the research and development of new drug. Chin. J. New Drug 15 (5), 328–334.

Mihmanli, M., Idiz, U.O., Kaya, C., Demir, U., Bostanci, O., Omeroglu, S., Bozkurt, E., 2016. Current status of diagnosis and treatment of hepatic echinococcosis. World J. Hepatol. 8, 1169–1181.

Nakamura, K., Uenaka, T., Nagasu, T., Sugumi, H., Yamaguchi, A., Kotake, Y., Okada, T., Kamata, J., Niijima, J., Taniguchi, T., Koyanagi, N., Yoshino, H., Kitoh, K., Yoshimatsu, K., 2003. A novel carbazole topoisomerase II poison, ER-37328: potent tumoricidal activity against human solid tumors in vitro and in vivo. Cancer Sci. 94, 119–124.

Nicolao, M.C., Elissondo, M.C., Denegri, G.M., Goya, A.B., Cumino, A.C., 2014. In vitro and in vivo effects of tamoxifen against larval stage *Echinococcus granulosus*. Antimicrob. Agents Chemother. 58, 5146–5154.

Pakala, T., Molina, M., Wu, G.Y., 2016. Hepatic echinococcal cysts: a review. J. Clin. Transl. Hepatol. 4, 39–46.

Pensel, P.E., Ullio Gamboa, G., Fabbri, J., Ceballos, L., Sanchez Bruni, S., Alvarez, L.I., Allemandi, D., Benoit, J.P., Palma, S.D., Elissondo, M.C., 2015. Cystic echinococcosis therapy: albendazole-loaded lipid nanocapsules enhance the oral bioavailability and efficacy in experimentally infected mice. Acta Trop. 152, 185–194.

Saulnier, M.G., Langley, D.R., Frennesson, D.B., Long, B.H., Huang, S., Gao, Q., Wu, D., Fairchild, C.R., Ruediger, E., Zimmermann, K., St Laurent, D.R., Balasubramanian, B.N., Byas, D.M., 2005. Novel 3', 60-anhydro and N12, N13-bridged glycosylated fluoroindolo [2, 3-a] carbazoles as topoisomerase I inhibitors. Fluorine as a leaving group from sp3 carbon. Org. Lett. 7, 1271–1274.

Spicher, M., Roethlisberger, C., Lany, C., Stadelmann, B., Keiser, J., Ortega-Mora, L.M., Gottstein, B., Hemphill, A., 2008. *In vitro* and *in vivo* treatments of echinococcus protoscoleces and metacestodes with artemisinin and artemisinin derivatives. Antimicrob. Agents Chemother. 52, 3447–3450.

Spiliotis, M., Brehm, K., 2009. Axenic in vitro cultivation of *Echinococcus multilocularis* metacestode vesicles and the generation of primary cell cultures. Methods Mol. Biol. 470, 245–262.

Stadelmann, B., Kuster, T., Scholl, S., Barna, F., Kropf, C., Keiser, J., Boykin, D.W., Stephens, C.E., Hemphill, A., 2011. *In vitro* efficacy of dicationic compounds and mefloquine enantiomers against *Echinococcus multilocularis* metacestodes. Antimicrob. Agents Chemother. 55, 4866–4872.

Teggi, A., Lastilla, M.G., De Rosa, F., 1993. Therapy of human hydatid disease with mebendazole and albendazole. Antimicrob. Agents Chemother. 37 (8), 1679–1689.

Wang, W., Sun, X., Sun, D., Li, S., Yu, Y., Yang, T., Yao, J., Chen, Z., Duan, L., 2016. Carbazole aminoalcohols induce antiproliferation and apoptosis of human tumor cells by inhibiting topoisomerase I. Chemmedchem 11 (24), 2675–2681.

Wang, W., Li, J., Yao, J., Wang, T., Li, S., Zheng, X., Duan, L., Zhang, W., 2017a. *In vitro* and *in vivo* efficacies of novel carbazole aminoalcohols in the treatment of cystic echinococcosis. J. Antimicrob. Chemother. 72, 3122–3130.

Wang, W., Li, Q., Wei, Y., Xue, J., Sun, X., Yu, Y., Chen, Z., Li, S., Duan, L., 2017b. Novel carbazole aminoalcohols as inhibitors of β-hematin formation: antiplasmodial and antischistosomal activities. Int. J. Parasitol. Drugs Drug Resist. 7 (2), 191–199.

Zheng, Q., Liu, C.S., Jiang, B., Xu, L.L., Zhang, H.B., 2013. Effect of long-term use of albendazole on mice liver. Chin. J. Parasitol. Parasit. Dis. 31 (3), 193–197.