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▣ BRIEF COMMUNICATION

In Vitro Effects of SB202190 on *Echinococcus granulosus*

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Abstract: Spillage of cyst contents during surgical operation is the major cause of recurrence after hydatid cyst surgery. Instillation of a scolicidal agent into a hepatic hydatid cyst is the most commonly employed measure to prevent this complication. SB202190 is a pyridinyl imidazole derivative and is known to be a specific inhibitor of p38 MAPK. In the present study, the scolicidal effect of SB202190 was investigated. Freshly isolated *Echinococcus granulosus* protoscolices were subjected to SB202190 treatment (10, 20, 40, and 80 μM), and the effects on parasite viability were monitored by trypan blue staining. Corresponding effects were visualized by scanning and transmission electron microscopy. Dose-dependent protoscolex death within a few days of SB202190 treatment was observed. Although the in vitro scolicidal effect of SB202190 was satisfactory, the in vivo efficacy of this drug and also possible side effects remain to be further investigated.

Key words: *Echinococcus granulosus*, hydatid cyst, protoscolex, SB202190

Cystic echinococcosis (CE) caused by the larval stage of *Echinococcus granulosus* is a life-threatening disease of serious public health concern. The disease is distributed worldwide, and affects humans as well as domestic livestock, including cattle, sheep, horses, and others [1].

Surgical removal of the intact hydatid cyst is the most preferred method of therapy for hydatidosis. One of the major surgical complications of hydatidosis is recurring (secondary) cystic echinococcosis after operation for primary hydatid disease. Dissemination of protoscolex-rich fluid during surgery is a major cause of recurrence and multiple secondary echinococcosis [2]. Instillation of scolicidal agents into a hepatic hydatid cyst is the most commonly employed measure to prevent this serious complication [3]. Currently, many scolicidal agents, which have some complications of their own, have been used for inactivation of the cyst content. Surgeons need less harmful and more effective scolicidal solutions for use in hydatid cyst surgery [4,5].

The p38 is a member of the mitogen-activated protein kinase (MAPK) family which includes the extracellular signal-regulated kinase (ERK1/2, ERK5, nd ERK8), the c-Jun N-terminal kinase (JNK1/2/3), and p38 kinase. The p38 MAPK is a key mediator in cytokine-induced signaling events that are activated in response to a variety of extracellular stimuli such as stress factors, apoptosis, and proliferation. Therefore, the p38 MAPK plays an integral role in disease states, suggesting that the p38 MAPK can serve as a potential therapeutic target for the treatment of many diseases. Inhibition of the protein kinases represents an attractive strategy for therapeutic intervention. In particular, the p38 MAPK inhibitors have been used in the in vitro and in vivo systems as well as in the clinical trials [6,7]. This is further supported by the fact that SB202190 and ML3403, inhibitors of p38 MAPK, led to dephosphorylation of EmMPK2 in *Echinococcus multilocularis* and effectively killed parasite vesicles [8]. In this study, we made use of this knowledge. We demonstrated that the activity of *E. granulosus* protoscolices can be blocked by SB202190, which is a specific inhibitor of p38 MAPKs.

Hydatid cysts from the liver and lungs of naturally infected sheep were obtained from an abattoir located in Shihezi of Xinjiang Province, China. Protoscolices were collected aseptically and washed several times with PBS (pH 7.2). These were placed into a culture medium (Dulbecco's minimal essential medium, 2 mM glutamine, 200 U/ml of penicillin, 200 mg/ml of streptomycin) supplemented with 10% FCS and phenol red. Cultures were kept in culture flasks placed in an upright position in an incubator at 37˚C, 5% CO_2, with medium changes every 3-5 days. Following the isolation of protoscoli-

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ces from hydatid cysts, the trypan blue dye exclusion test revealed that >95% of the parasites were still viable, and >65% of protoscolices exhibited distinct movements.

Treatment of protoscolices was initiated within 10 days of in vitro culture. Three T25 tissue culture flasks containing at least 500 protoscolices in 20 ml culture medium were supplemented with 10, 20, 40, and 80 μM SB202190. Control cultures were supplemented with equal volumes of DMSO alone. The viability of protoscolices was assessed on a daily basis by microscopic observation of movements, flame cell activity, and trypan blue exclusion test. Moreover, other criteria, such as muscular movements, morphological integrity of the whole body, and motility of flame cells, were taken into accounts to confirm the viability. The corresponding numbers of viable/non-viable protoscolices were determined in 10 randomly chosen fields by phase contrast microscopy. Each experiment was repeated 3 times.

SB202190 exhibited profound activities on protoscolices at 80 μM, in that they reduced the number of viable protoscolices by 70% within a period of 1 day. At 40 μM, SB202190 had a clearly decreased efficacy, with 40% of protoscolices still viable, compared to 80 μM, which left a small portion of parasites viable, after 2 days of treatment. Whereas SB202190 at 20 μM and 10 μM were clearly less efficient than SB202190 at 40 μM and 80 μM. The number of dead protoscolices increased with time. At day 5, only a small part of parasites could be seen viable in cultures treated with 80 μM of SB202190. In contrast, parasites cultured in the absence of SB202190 were not significantly altered (Fig. 1).

To visualize the structural alterations in protoscolices imposed by SB202190 treatment, parasites were processed for scanning (SEM) and transmission electron microscopy (TEM) after the initiation of treatment with 40 μM SB202190. Control cultures exhibited no ultrastructural alterations in parasite tissue during the whole incubation period (Figs. 2A, 3A). In contrast, morphological and ultrastructural damages were detected in treated protoscolices. At day 3 post-incubation, observations by SEM and TEM of protoscolices incubated with SB202190 revealed the ultrastructural changes. The ultrastructural changes included tegumental alterations (Fig. 2B), rostellar disorganization, loss of hooks, and shedding of microtriches of the scolex region (Fig. 2C). In some protoscolices, loss of morphology was evident (Fig. 2D). Ultrastructural studies by TEM revealed severely affected internal tissues, resulting in loss of its integrity, an increase in the number of lipid droplets, and an increase of vacuoles (Fig. 3B, C).

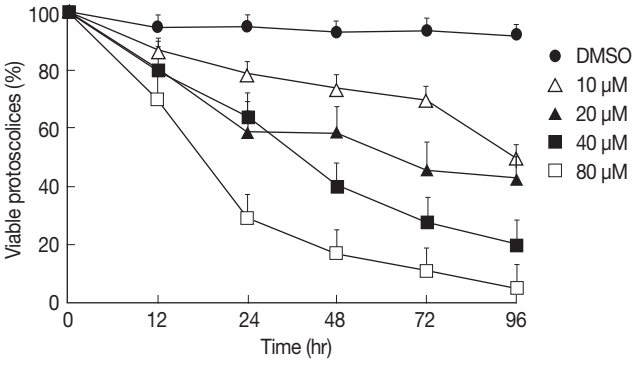

Fig. 1. Loss of viability of *E. granulosus* protoscolices during in vitro SB202190-treatment. Viability was determined through trypan blue staining. Note the dose-dependent effect of SB202190.

The aim of this study was to investigate the efficacy of p38 MAPK inhibitor against the cestode parasite *E. granulosus*, the causative agent of CE. In vitro culture of protoscolices was used to demonstrate the parasiticidal effects of SB202190 on protoscolices.

Surgery is still the first treatment of choice for symptomatic cases of CE. However, surgery has been associated with local recurrence or secondary dissemination [9]. Avoiding spillage of the cyst contents and the use of effective scolicidal agents are essential to lower the recurrence rate.

To date, many scolicidal agents have been used for inactivation of the hydatid cyst content. Many of these scolicidal agents may cause unacceptable side effects. Hypertonic saline (20%) has been found to be 100% effective against scolices of hydatid cyst after 15 min, but acute hypernatremia, leading to convulsions, intracranial bleeding, necrosis, and myelinolysis, has been reported for this agent [10]. Ethyl alcohol (95%) was found to be 100% effective after 15 min in undiluted form. Further dilutions of 47% and 9.5% ethyl alcohol were ineffective as a scolicidal agent after 5 and 10 min exposures [11]. Moazeni and Larki [5] investigated the in vitro scolicidal effects of highly acidic and alkaline solutions. They reported a 100% scolicidal effect for solutions with pH 1.0 and 14.0 after 5 min. In their study, the scolicidal activity of acidic solutions with pH 2.0, 3.0, and 4.0 after 10 min was 100%, 100%, and 21.5%, respectively. The scolicidal activity for alkaline solutions with pH 13.0, 12.0, and 11.0 was 99.7%, 33.4%, and 24.5%, respectively. A clinical study showed that intracystic injection of 0.04% Chx-Glu was effective on both protoscolices and the germinative membrane during hydatid cyst surgery [4].

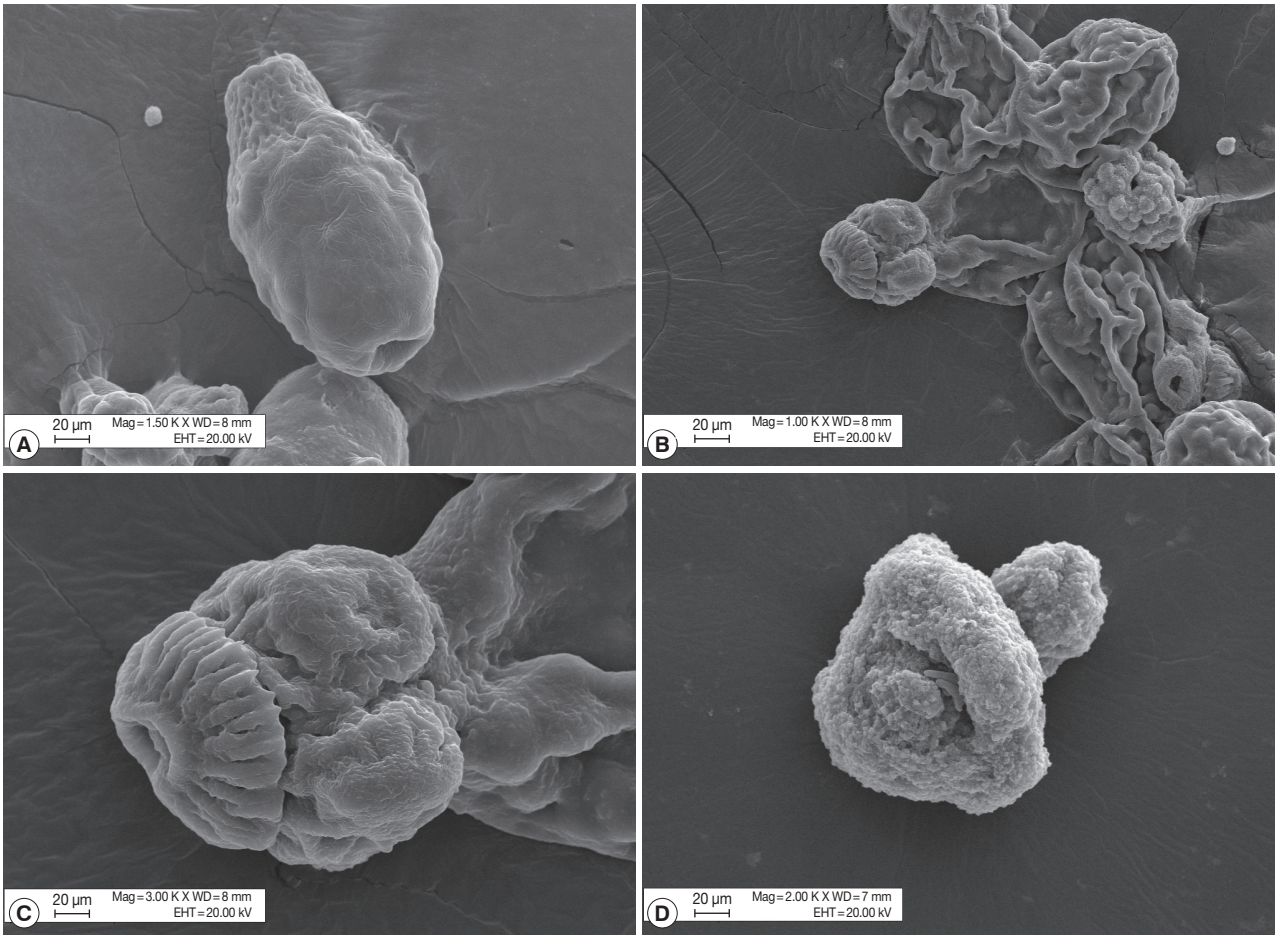

Fig. 2. Scanning electron microscopy of *E. granulosus* protoscolices cultured for 3 days in the presence or absence of SB202190 (40 μM). (A) Protoscolices cultured in medium containing DMSO (1:1,000). (B-D) Protoscolices cultured in the presence of SB202190. Note the extensive drug-induced damages.

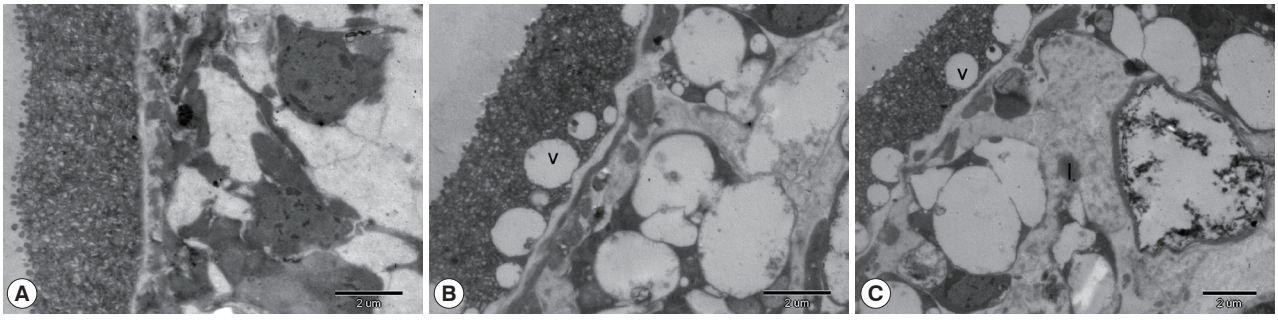

Fig. 3. Transmission electron microscopy of *E. granulosus* protoscolices treated with DMSO (A) or 40 μM SB202190 (B, C) for a period of 3 days. Note the presence of vacuoles (v) and lipid droplets (l).

Scientific reports have shown that p38 inhibitors have many properties. The p38 MAPK inhibitor can serve as a potential therapeutic target for the treatment of not only inflammatory diseases but also cancer. Paillas [12] has suggested that SB202190 enhances the efficacy of irinotecan-based chemotherapy in non-responder colorectal cancer patients. Brumlik [13] has demonstrated that human p38 MAPK inhibitors reduced in vitro and in vivo replication of the protozoan parasites *Toxoplasma gondii* and *Encephalitozoon cuniculi*. In a recent investigation, Gelmedin [8] has demonstrated that pyrimidyl imidazole

compounds, originally designed to inhibit mammalian p38-like MAPK isoforms, can also inhibit EmMPK2 and are effective in killing in vitro cultivated parasite vesicles at concentrations that do not affect cultured mammalian cells. Therefore, it is useful to test the effect of p38 MAPK inhibitor on *E. granulosus* protoscolices, with the aim of a more potent antiparasitic activity and reduction of toxicity.

SB202190 is a pyridinyl imidazole derivative and is known to be a specific inhibitor of p38 MAPK. In line with these studies, we have proved that SB202190 is effective in inactivating in vitro cultured *E. granulosus* protoscolices. Moreover, the increased effectiveness was induced proportionally with increased concentrations of SB202190. Protoscolices cultured with 80 µM SB202190 were killed considerably faster than protoscolices cultured with 40 µM, 20 µM, and 10 µM SB202190. After 1 day of exposure to 80 µM SB202190, viability was approximately 30%, and it was reduced to 5% after 4 days of incubation. As demonstrated in our experiments, the incubation of drug produced ultrastructural alterations with tegumental alterations, rostellar disorganization, and loss of hooks, and the internal tissue was severely affected. In vitro SB202190 did show some potential as a scolicidal agent for inactivation of the hydatid cyst.

In conclusion, we demonstrated the in vitro efficacy and parasiticidal activity of SB202190 against *E. granulosus* protoscolices. In the next step, we will investigate the efficacy of SB202190 in an animal model, where the active compound has to reach the infected organ and the parasitic tissue at a sufficiently high concentration.

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