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Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2016.06.013

In vitro anti-hydatic and immunomodulatory effects of ginger and [6]-gingerol

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ARTICLE INFO

Article history:
Received 15 May 2016
Received in revised form 16 Jun 2016
Accepted 21 Jun 2016
Available online 29 Jun 2016

Keywords:
Ginger
[6]-Gingerol
Echinococcosis
Cytotoxic activity
Nitric oxide

ABSTRACT

Objective: To study *in vitro* anti-hydatic and immunomodulatory effects of ginger and [6]-gingerol as an alternative therapy for cystic echinococcosis.
Methods: Effect of a commonly used herbal product and ginger (*Zingiber officinale*) towards protoscoleces (PSC) and cyst wall *in vitro* was studied. The effect of [6]-gingerol, and the pungent constituent of ginger, was also evaluated on PSC culture. Furthermore, the activity of both extracts in association with interferon-gamma (IFN-γ) on PSC co-cultured with mononuclear cells of hydatic patients was evaluated. The nitric oxide (NO) production was measured in each co-culture.
Results: Ginger exhibited a concentration- and time-dependent cytotoxic effect against PSC and cyst wall. Interestingly, ginger was more effective than the [6]-gingerol. Moreover, additional parasitic effect between extracts and IFN-γ are also observed in co-cultures. Furthermore, both extracts attenuated the NO production elicited by this infection or by the IFN-γ.
Conclusions: Ginger has an important anti-hydatic effect *in vitro*. This effect is amplified in the presence of IFN-γ. Moreover, this herbal product may protect against host's cell death by reducing the high levels of NO. Ginger may act, at least, through the [6]-gingerol. All our data suggest the promising use of ginger in the treatment of *Echinococcus granulosus* infection.

1. Introduction

Cystic echinococcosis is a zoonotic disease in humans and sheep. This infection is caused by the larval stage of *Echinococcus granulosus* (*E. granulosus*). Man is being infected after ingestion of disseminated eggs in water, plants or on over the definitive hosts (dogs). Each egg, containing an oncosphere, gives a unilocular cyst especially in the liver and lungs. Surrounded by a thin wall: cyst wall (CW), the cyst is filled with fluid containing brood capsules and protoscoleces (PSC).

The development of hydatic cyst for a long period induces many complications like organ malfunction and can lead to death [1]. The preferred therapy is cyst removal by surgery. However, the protoscoleces dissemination can occur during surgery leading to a high frequency of relapse. Chemotherapy with benzimidazole carbamate derivatives, amphotericin B and praziquantel is also used. This is the only option in many inoperable cases like cyst in brain or multiple cysts and in the immune-depressed host. Nevertheless, these drugs are not efficient in some cases and leads to liver toxicity and other side effects [2]. All these data prompted us to find new drugs for therapy.

The natural products are attracting attention as new therapeutic drugs against a number of diseases. Among their effects we found antiparasitic and antioxidant activities [3]. One of commonly used herbal product in traditional medicine all over the world is ginger (*Zingiber officinale*) [4,5]. There are few studies about the direct effect of this rhizome or other natural products against the helminthes. Some of these studies are related to *E. granulosus* and *Echinococcus multilocularis* using ginseng derivates or other herbs or natural products [6]. However, as far as we know, there are no studies about the effect of ginger on *E. granulosus* metacestode in culture or coculture with mononuclear cells (PBMC) of hydatic patients.

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Peer review under responsibility of Hainan Medical College.
Foundation project: This work was supported by National Project in Health (PNR-Santé-2011-2014).

In this sense, we assessed here the effect of crude aqueous extract of dried ginger on *E. granulosus* CW and PSC in culture. Furthermore, we evaluated the activity of this extract in association with IFN-γ on PSC viability and nitric oxide (NO) production in coculture and culture performed with PBMC of hydatic patients and healthy donors. The effects of ginger were also compared with those of [6]-gingerol, one of the active components of ginger.

2. Materials and methods

2.1. Ginger and [6]-gingerol

The dried rhizome of *Zingiber officinale* Roscoe (family Zingiberaceae), was procured from a local market in Algiers, Algeria and then has been identified and authenticated by a botanist. The bioactive compound [6]-gingerol was obtained from Calbiochem (San Diego, CA).

2.2. Preparation of ginger crude aqueous extract and [6]-gingerol

The crude aqueous extract was prepared by dissolving 1 mg of the dried extract of ginger in 1 ml of RPMI-1640 medium supplemented with 5% of heat-inactivated FCS (fetal calf serum, sigma) and antibiotics. Tubes were then mixed and centrifuged. The aqueous extract was then filtered through 0.22 μm (Millipore) and stored at −20 °C until use.

The [6]-gingerol was diluted from the stock solution (dissolved in DMSO) with the culture medium (RPMI-1640) at the maximum final concentration of DMSO at 0.1%.

2.3. Patients

The peripheral blood samples were obtained from twenty Algerian patients carrying hydatic cyst in lungs (n = 20) at Department of Surgery, Mustapha Bacha Hospital, Algiers, Algeria. The blood samples were obtained before (no more than 1 week) and after (24–72) h surgery. All patients having other infection or inflammatory chronic diseases were excluded from this study. Blood samples from healthy donors (n = 10, from the same region of Algeria) were included in this study as healthy controls. All samples were used immediately after collection for PBMC preparation and culture.

All participants (patients and healthy donors) gave informed consent for this study, which was in accordance with the guidelines of the ethic committee of the Algerian agency of research and development in health (ATRSS). All procedures were performed in accordance with Helsinki declaration of 2008.

2.4. Preparation of PSC and CW

The hydatid cysts (n = 5) were obtained after surgery in lungs and livers at Mustapha Bacha Hospital, Algiers, Algeria. Cysts were washed several times with sterile PBS, pH 7.4 supplemented with antibiotics.

CW was prepared as previously described [7]. Briefly, cyst wall, obtained after hydatid fluid removal, was washed several times with sterile PBS. This membrane was then cut into small pieces (1 cm × 1 cm) and washed again. CW was finally maintained in RPMI-1640 medium supplemented with 5% of heat-inactivated FCS and antibiotics until culture.

PSC were prepared as previously described [8,9]. Briefly, free PSC were obtained by centrifugation of hydatid fluid followed by washing in sterile PBS. The viability of PSC was determined prior the cultures by microscopic examination using 0.1% eosin as a vital stain. While viable PSC still unstained, dead PSC become stained. The appropriate percentage of viability was 95% or more. PSC were then maintained in RPMI-1640 medium supplemented with 5% of heat-inactivated FCS until culture and coculture.

2.5. CW and PSC culture and treatment

Two pieces of CW and 2×10^3 viable PSC were placed in each well of 24-well plastic trays. They were cultured in RPMI-1640 medium supplemented with antibiotics and 10% heat-inactivated FCS and treated with different concentrations of ginger (100 μg/mL for CW and 1, 10 and 100 μg/mL for PSC). PSC cultures were also treated with [6]-gingerol (100 μg/mL). The untreated cultures were used as a negative control. PSC treated with 0.1% DMSO vehicle without [6]-gingerol were also considered as a control. Cultures were then incubated at 37 °C in a humid atmosphere and 5% CO_2 for 72 h for CW and 24 h and 48 h for PSC.

Cultures were observed under microscope before and after incubation. Percentages of viable protoscoleces were determined by microscopic examination using 0.1% eosin as a vital stain.

2.6. PBMC culture and coculture with PSC

PBMC were prepared from blood of hydatic patients and healthy donors as previously described [8]. Briefly, PBMC isolated by density gradient were washed three times with sterile PBS. Cell viability was checked using 0.2% trypan blue dye. The appropriate percent of viable cells was always >98%. Viable of 10^6 cells/mL were then resuspended in RPMI-1640 medium supplemented with antibiotics and 10% of FCS.

Cells were immediately cocultured with PSC and stimulated with 100 μg/mL of ginger or [6]-gingerol alone or with IFN-γ (100 U/mL). The unstimulated cocultures were used as negative control. The cocultures treated with 0.1% DMSO vehicle without [6]-gingerol were also considered as a control. Cells were also stimulated with ginger extract (1, 10 and 100 μg/mL) and [6]-gingerol (100 μg/mL).

The effects of ginger and [6]-gingerol on nitrites production was demonstrated using L-NMMA (0.5 mM), (Sigma) and human recombinant IFN-γ (100 U/mL) [8] (HuIFN-γ is a gift from Dr. J. Wietzerbin, INSERM U365 – 'Interférons et cytokines' – Institut Curie, INSERM U932 – 'Immunité et cancer' – Institut Curie, Paris, France).

Cocultures and cultures were then incubated at 37 °C in humid atmosphere and 5% CO_2. After 20 h, percentages of viable protoscoleces were determined in coculture as described previously in Section 2.4. Moreover, nitrites production was evaluated in coculture and culture supernatants.

2.7. Nitrites measurement

Nitric oxide production was evaluated by measuring nitrites concentration (NO_2^-) in each supernatant by Griess method as