THE EFFECT OF HYDROALCOHOLIC AND KETONIC EXTRACTS OF Zingiber officinale RHIZOMES ON THE NEUTROPHIL MIGRATION INDUCED BY BCG IN MICE THORACIC CAVITY

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Abstract- Plant extracts have been used for centuries as a popular manner to treat several health disorders. During the last ten years the study of those extracts has attracted the attention of pharmacologists due to the necessity of new drugs discovery to old pathologies. Ginger, the rhizome of Zingiber officinale Roscoe (Zingiberaceae) is a common constituent of diet worldwide and it has been reported that its extracts present some pharmacological activities. Here we investigate the effects of two different crude extracts, hydroalcoholic and ketonic, of ginger rhizomes on the model of BCG-induced mice pleurisy. Our results demonstrated that both extracts of Zingiber officinale were able to significantly reduce inflammatory cells migration to pleural cavity in response to BCG injection. However, the ketonic extract demonstrated the most powerful effect in reducing cell migration.

Introduction

In 1882 Robert Koch identified the tubercle bacillus as the etiologic agent of an infectious disease which was a very important cause of death at that time, in adults living in Europe. Today, more people die from Mycobacterium tuberculosis infection than from any other pathogen. It is assumed that 60 million people suffer from active tuberculosis, and that about one third of the total world population is infected with M. tuberculosis. In Brazil, 100,000 new cases of tuberculosis are reported annually (Inge et al., 1993; Arruda et al., 1998).

The human immune response against tuberculosis is mainly controlled by cellular-mediated immunity by activated T-cells (Kaufmann, 1995), leading to augmented mycobacterical macrophage mechanism through the production of IFN-gamma. The occurrence of multidrug resistant strains and the expanding AIDS threat make the problem of tuberculosis more problematic, and the searching for new drugs is of increasing interest. The resurgence of tuberculosis in developing nations, combined with the magnitude of the epidemic throughout the rest of the world, has made it imperative to design a cohesive strategy for the prevention, treatments and research of new drugs (Kaplan and Freedman, 1996). In this context, plant materials may constitute new sources of drugs to threat tuberculosis. In the last ten years the study of plant extracts has increasing attracted the attention of pharmacologists because of the discover of new drugs for old pathologies including infectious diseases.

Ginger, the rhizome of Zingiber officinale Roscoe (Zingiberaceae), originally from south-east Asia, is found in South America and several countries of the African continent and is a common constituent of diet worldwide (Sertié et al., 1992). Recently we demonstrated an important antiinflammatory effect of ginger using the classical model of carrageenan-induced rat paw edema and rat skin edema induced by different substances (Penna et al., 2003). In the present work we investigated the anti-inflammatory activity of ketonic and hydroalcoholic extracts of Zingiber officinale in the animal model of BCG-induced mice pleurisy.
Materials And Methods

Animals- All experiments were carried out in accordance with the guidelines of Oswaldo Cruz Foundation for animal care. The experiments were carried out on Male C57I/B6 mice weighing between 20 and 25 g kept at standard conditions of temperature (22-25°C), relative humidity (40-60%) and light/dark cycle, with food and water “ad libitum”. The animals were provided by Central Biotery of Oswaldo Cruz Foundation (FIOCRUZ). All animals were placed in a common box and randomly divided into groups of six animals

Hydroalcoholic and Ketonic extracts of Z. officinale - Z. officinale extract was obtained as previously described (Sertié et al. 1992). Z. officinale rhizomes were collected in March (2002) at Caraguatatuba, State of São Paulo and identified by the herbarium staff of Department of Botany, Biosciences Institute - University of São Paulo (SP), Brazil. The pharmacological trials were carried out with the dry material dissolved in 0.9% saline.

Induction of mice pleurisy

Pleurisy was induced by the intrathoracic (i.t.) injection of 100 µl (8 X 10^6 bacilli) of the agonist solution (BCG) An equal volume of sterile saline was injected into the controls.

The treatments of mice with Z. officinale extracts were always performed by intraperitoneal injections of different doses of both extracts 30 minutes after BCG injection. The extracts were always injected at the same volume of 0.5 ml. Total Leukocyte Counts

After four hours of the BCG injection the animals were killed with CO₂ inhalation. The thorax was immediately opened and the pleural cavity was washed with 1 ml of (PBS) plus heparin (20 IU.ml-1) and the total volume was harvested. The total cell count in the pleural cavity was determined using a Neubauer chamber. The results are given as millions of cells per cavity.

Differential Leukocyte Counts

The exudate smears were stained with May-Grünwald-Giemsa for differential cell count which was performed with a light microscope using immersion objective. The numbers of eosinophils, neutrophils and Mononuclear cells were determined

Chemicals and Reagents

BCG, moureau strain, from the Ataulpho de Paiva Foundation, Rio de Janeiro, was generously donated by Dr. M.G.M. Henriques (Oswaldo Cruz Foundation) Brazil. The number of mycobacteria used in all assays was 8 X 106 bacilli as described in the literature (Silva et al., 1985; Tomioka et al., 1996).

Statistical Analysis

The data are expressed as mean ± standard error of the mean (SEM) and were statistically evaluated by analysis of variance (ANOVA), followed by the Newman-Keuls-Student test. In the case of comparison between two groups, the analysis was performed by unpaired Student’s t-test; p<0.05 was considered to be significant. Each experimental group of inbred mice contained six animals.

Results

Effect of Hydroalcoholic Extract of Zingiber officinale on the BCG-induced mice pleurisy

The intrathoracic (i.t.) injection of BCG in mice stimulated intense leukocyte accumulation within 4 h. The i.t. injection of BCG in mice stimulated a significant neutrophil accumulation in the pleural cavity within 4 h (Fig. 1). The intraperitoneal administration of hydroalcoholic extract of Zingiber officinale significantly reduced the total number of neutrophils (51 ± 4, 38 ± 6 and 79 ± 9 %) at the doses of 20, 60 and 180 mg/kg, respectively.

Figure 1: The effect of intraperitoneal administration of hydroalcoholic root extract of Zingiber officinale (Zo) on the Number of Neutrophils in the BCG-induced mice pleurisy. Results are expressed as mean ± S.E.M. from six animals. The abscissa represent the number of cells at the 4th hour time point.

Effect of Ketonic Extract of Zingiber officinale on the BCG-induced mice pleurisy

The intraperitoneal administration of Ketonic extract of Zingiber officinale significantly reduced the total number of leukocytes. Concerning on neutrophils, intrathoracic BCG stimulated intense accumulation in the pleural cavity in the 4 h period (Fig. 2). The intraperitoneal administration of Ketonic extract of Zingiber officinale reduced very significantly the total number of neutrophils (98 ± 4, 79 ± 11, 77 ± 8 %) at the doses of 20, 60 and 180 mg/kg, respectively..
Discussion

The human immune response against tuberculosis is mainly controlled by cellular-mediated immunity by activated T-cells (Kaufmann, 1995), leading to augmented mycobactericidal macrophage mechanisms through the production of IFN-γ (Flynn et al., 1993). Tuberculous pleurisy is considered a benign clinical condition, since patients clear the infection even in the absence of treatment (Stead et al., 1955). In this condition the pleural compartment contains an exudate rich in inflammatory cells, as well as immune (Barnes et al., 1993) and non-immune soluble factors (Costa et al., 1995). While tuberculosis represents a threatening disease caused by intracellular bacteria, our understanding of the cellular and molecular interactions between mycobacteria and host cells is far from complete. The initial events during a primary pulmonary infection with M.tuberculosis complex (MCT) are poorly understanding and there are few models to evaluate the sequence of events that follows the first contact of the host with the mycobacteria. In this paper we used the pleural cavity of the mouse because it is a straightforward and well-established model (Henriques et al., 1990, 1996, Bozza et al., 1994, Menezes-de-Lima-Junior et al., 1997). The injection of M.bovis BCG into mouse pleural cavity induces an intense biphasic inflammatory reaction that peaks at 24h and 15 days. At 4h occurs an influx of neutrophils that is maximal at 24h. An intense influx of eosinophils and mononuclear cells. Another leukocyte influx is observed at 15 days comprising by mononuclear cells and some neutrophils.

In the present work we could observe that intrathoracic injection of BCG was able to induce a significant influx of leukocytes to the mice pleural cavity in 4 hours. The intraperitoneal administrations of ketonic and hydroalcoholic extracts of Z. officinale 30 minutes after BCG injections were able to significantly reduces the leukocyte migration with a special emphasis to the effect observed on neutrophils. Neutrophils are the predominant leukocytes to arrive as sites of acute inflammation, capable of defending the host against bacterial infections. Neutrophils accumulation is also intimately associated with edema formation and the recruitment of other phagocytic leukocytes such as monocytes (Nourshargh, 1993).

Differences concerning the pharmacological properties of hydroalcoholic and ketonic extracts of Z. officinale has been reported. Sharma et al., (1997) observed that anti-emetic activity of Z. officinale differs significantly to aqueous, ethanolic or ketonic extracts. The authors found that aqueous extract was ineffective in protecting against cisplatin-induced emesis, while ethanolic and ketonic extracts were able to prevent emesis. However, the ketonic extract was clearly more effective preventing this cisplatin-induced emesis.

Previous studies from our group demonstrated the anti-inflammatory effect of the hydroalcoholic extract of Zingiber officinale rhizomes on rat paw and skin edema (Penna et al., 2003). In this previous work we suggested that the antiedematogenic activity of the extract seemed to be related, at least in part, to an antagonism of the serotonin receptor, although the cyclooxygenase pathway should also be involved because the antiedematogenic effect of the extract in the carrageenan-induced rat paw edema was observed from the first hour of the reaction, when the cyclooxygenase products are classically mediating the phenomenon. As mentioned in the introduction, other authors reported important anti-inflammatory effects of Z. officinale extracts and products. Sharma et al., (1994) reported that eugenol and ginger oil have potent anti-inflammatory and/or antirheumatic properties using a model of severe chronic adjuvant arthritis in rats induced by Mycobacterium tuberculosis bacilli in liquid paraffin. Patients with pulmonary tuberculosis develope pleural effusions with a high protein content. BCG enhances pleural mesothelial cell (PMC) release of vascular endothelial growth factor (VEGF) and dow-regulates beta-catenin (adherens junction protein) expression leading to increased permeability across mesothelial monolayer (Mohammed et al., 2003). Its well established that beta-catenin expression is closely related to prostanoids, but the linking between BCG infection, prostanoids and beta-catenin is missing.

In conclusion, our results clearly demonstrates that Z. officinale ketonic and alcoholic extracts were able to significantly inhibit leukocyte migration to the mice pleural cavity.
induced by intrathoracic BCG injection with emphasis to the effect of the ketonic extract on neutrophil migration. Considering this important observed activity, *Zingiber officinale* could represent a potential source of new compounds in the future, helping to treat tuberculosis.

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**References**


