European Journal of Pharmacology 608 (2009) 76–83

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Immunopharmacology and Inflammation

Chemotherapeutic potential of two gallic acid derivative compounds from leaves of *Casearia sylvestris* Sw (Flacourtiaceae)

Saulo L. Da Silva [a,*], Jamal da S. Chaar [b], Tomomasa Yano [c]

[a] Centro de Ciências da Saúde, Universidade Federal de São João Del Rei, UFSJ, 35501-296, Divinópolis, MG, Brazil
[b] Instituto de Ciências Exatas, Universidade Federal do Amazonas, UFAM, 69077-000, Manaus, AM, Brazil
[c] Instituto de Biologia, Universidade Estadual de Campinas, UNICAMP, 13081-970, Campinas, SP. Brazil

ARTICLE INFO

Article history:
Received 13 June 2008
Received in revised form 20 January 2009
Accepted 9 February 2009
Available online 15 February 2009

Keywords:
Casearia sylvestris
MTT
Ehrlich ascites tumor
Lewis lung carcinoma
Gallic acid

ABSTRACT

Casearia sylvestris is a plant used in the treatment of several diseases, including cancer. Studies have shown that *C. sylvestris* presents an interesting antitumoral potential, due to the presence of casearins and some sesquiterpens with antitumoral activity. In this work, we tested the potential chemotherapeutic of two gallic acid-derived compounds isolated from *C. sylvestris* leaves: isobutyl gallate-3,5-dimethyl ether (IGDE) and methyl gallate-3,5-dimethyl ether (MGDE). We utilized two tumoral models: Ehrlich ascites tumor cells (EAT)/BALB/c mice and Lewis lung cancer cells (LLC1)/C57bl/6 mice. MGDE and IGDE increased the survival of mice inoculated with EAT cells and decreased the tumor volume in the LLC1 model, compared to control groups. Both compounds presented similar and low in vitro cytotoxicity against Ehrlich ascites tumor cells and did not present any significant toxicity against Lewis lung cancer cells. Since the direct in vitro activity against Ehrlich tumor and Lewis lung cancer cells was low, we investigated the effects of MGDE or IGDE treatment on the activity of total natural killer cells from Ehrlich ascites tumor-bearing mice, as a possible explanation for the mechanisms of these compounds in vivo. MGDE and IGDE improved NK cell cytotoxicity against Ehrlich ascites cells. As expected, tumor growth in non-treated mice markedly suppressed NK cell cytolysis while, IGDE completely reversed this effect, when mice were treated with 0.5 mg/kg dosages of these compounds for 4 days. The pharmacokinetic studies showed that IGDE remains in the organism for a long period of time, possibly explaining the higher compound efficiency.

1. Introduction

Casearia sylvestris Sw (Flacourtiaceae) is a plant popularly known as guaçatonga, chá-de-bugre or cafezinho-do-mato and is geographically distributed throughout Latin America (Lorenzi and Matos, 2002), occurring throughout the entire Brazilian territory extension (Hack et al., 2005; Barbosa et al., 2005). The Brazilian Karajá Indians prepare a bark maceration to treat diarrhoea and the Shipibo-Conibo Indians of Peru use a decoction of the bark for the treatment of diarrhoea, chest colds and flu. Other Indian tribes in Brazil mash the roots or seeds of *C. sylvestris* to treat wounds and, topically, leprosy (Taylor, 2002). Additionally, indigenous people throughout the Amazon rainforest utilize guaçatonga as a snakebite remedy (Mors et al., 2000; Alves, 2000, Da Silva et al., 2008a; Da Silva et al., 2009). A number of studies in the literature corroborate some of the medicinal properties of *C. sylvestris*, such as analgesic (Simões et al., 2001), cytotoxic (Da Silva et al., 2008a), anti-inflammatory (Raslan et al., 2002), antiulcerous (Basile et al., 1990; Esteves et al., 2005), antiproliferative (Mans et al., 2000) and antimicrobial (Da Silva et al., 2008b,c) properties.

The cytotoxic activity of plants from the genus *Casearia* has already been investigated. Mosaddick et al. (2004) demonstrated the cytotoxic activity of *C. costulata*, *C. grewiifolia*, *C. grayi* and *C. multinervosa* on P388 lymphoblast mice cells. Orberlies et al. (2002) showed that alcohol extracts of *C. sylvestris* are cytotoxic against macrophages J774; however Maistro et al. (2004) showed that the alcohol extracts of *C. sylvestris* were not genotoxic against HTC (hepatoma tissue cells) and VT79 (Chinese hamster lung) cells. Esteves et al. (2005) determined the composition of the essential oil obtained from *C. sylvestris* leaves and verified that the major component is bicyclogermacrene (40.9%), a component with low antitumoral potential. Nevertheless, a high percentage of this essential oil (around 17.5%) is constituted by two sesquiterpens, which present well-characterized cytotoxic activities: β-caryophyllene (13.8%) and α-humulene (3.7%) (Sylvestre et al., 2006; Hou et al., 2006; Xiao et al., 2006). In addition, Da Silva et al., (2008a) showed that the volatile oil of *C. sylvestris* is cytotoxic against A-549, HeLa and HT-29 (tumoral line cells), but not against macrophages and Vero cells (non-tumoral line cells). Recently, two gallic acid-derived compounds were isolated from the alcoholic extract produced from *C. sylvestris* leaves and were then

* Corresponding author. Rua Fortunato Badan, 103, Jardim Silvânia, Moji Mirim, SP, CEP 13806-679, Brazil. Tel.: +55 92 36392600; fax: +55 92 36474027.
E-mail address: biomol2@hotmail.com (S.L. Da Silva).

0014-2999/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2009.02.004

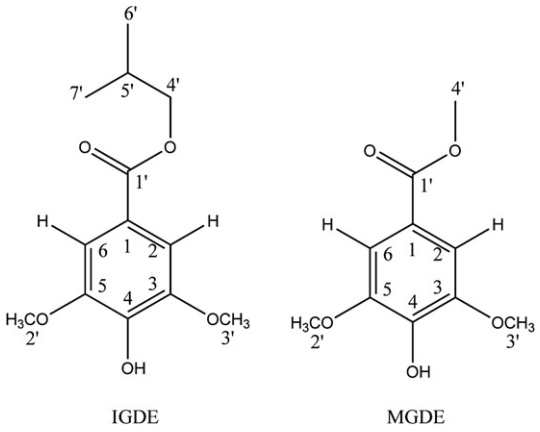

Fig. 1. Structures of isobutyl gallate-3,5-dimethyl ether (IGDE) and methyl gallate-3,5-dimethyl ether (MGDE), isolated from *C. sylvetris* leaves.

characterized (isobutyl gallate-3,5-dimethyl ether (IGDE) and methyl gallate-3,5-dimethyl ether (MGDE)—Fig. 1) (Da Silva et al., 2008a).

Gallic acid, as well as other polyphenolic substances, is a compound able to inhibit the growth of cells from several types of tumor (Saleem et al., 2002; Zhang et al., 2005). Hydroxybenzoic acids, such as gallic and ellagic acids, cause apoptosis in human DU-145 prostate cancer and HL-60 cells (Veluri et al., 2006; Lansky and Newman, 2007). In addition, other studies have shown that plants with abundant polyphenolic compounds are able to act not only on the humoral immune response (Kong et al., 2004), but also on the cell response, activating B lymphocytes with subsequent *in vitro* proliferation of T cells (Lin et al., 2005).

The aim of this study is to verify the chemotherapeutic potential of the IGDE and MGDE compounds. Therefore, the *in vivo* and *in vitro* antitumoral activities of both compounds extracted from *C. sylvestris* leaves were evaluated using a non-syngeneic ascites tumor model (Ehrlich ascites tumor cells/BALB/c mice) and other solid syngeneic human tumor model (Lewis lung cancer cells (LLC1)/C57bl/6 mice). Total natural killer cell (NK cell) activity in normal and Ehrlich ascites tumor-bearing mice was also examined in an attempt to determine a possible role of the immune system in its *in vivo* anti-tumor activity. The Ehrlich ascites tumor is an extremely aggressive and rapidly growing carcinoma, which is widely used to investigate the anti-tumor properties of several new agents. Progressive Ehrlich tumor growth is characterized by a high rate of glutamine consumption and profound alterations in the immune response (Lobo et al., 2000), which have been associated with metabolic alterations leading to decreased immunocompetence (Justo et al., 2000; Justo et al., 2003; Da Silva et al., 2007). For complementation of the experimental methods and for National Cancer Institute (NCI)'s Division of Cancer Treatment's recommendation, we also tested a syngeneic model utilizing Lewis lung carcinoma cells on C57bl/6 mice. The National Cancer Institute recommends (since 1976) the use of solid syngeneic models as a testing tool for new chemotherapic drugs, since many substances with a possible therapeutic efficiency can respond to ascites tumors but not to solid tumors. In other cases it is possible to observe an opposite effect (Talmadge et al., 2007).

2. Materials and methods

2.1. Plant material

Fresh leaves of *C. sylvestris* were collected in the city of Campinas, S.P., Brazil and a voucher was deposited in the herbarium of the Biology Institute of UNICAMP (State University of Campinas—Campinas, Brazil) under the number UEC 118743. The ethanol extract was produced by blending 500 g of fresh, washed and stirred leaves with 1000 ml of ethanol (analytical grade), for 15 min at room temperature and then filtering with analytical filter paper. The alcohol extract was centrifuged at 30 000 g for 15 min and the supernatant was concentrated to a semisolid paste using a rotavapor. This paste was lyophilized and then 5.6 g of a light green powder was obtained and stored at −20 °C.

2.2. Extraction of IGDE and MGDE

The complete process of extraction and characterization of isobutyl gallate-3,5-dimethyl ether (IGDE) and methyl gallate-3,5-dimethyl ether (MGDE) has been described by Da Silva et al. 2008a). Basically, this technique consists of the utilization of two chromatographic steps: Sephadex LH-20 column chromatography: The powder obtained from ethanol extract was dissolved in methanol and applied to a column packed with Sephadex LH-20 and eluted with methanol. The fractions were collected in test tubes and their absorbances read at 280 nm. Solvent was evaporated under vacuum at 40 °C. Dried fractions were stored in tinted glass bottles at −20 °C until use. Analytical and preparative Reverse Phase High-Performance Liquid Chromatography (RP-HPLC): The fraction eluted from Sephadex LH-20 column chromatography was submitted to Reverse Phase High-Performance Liquid Chromatography (RP-HPLC). Conditions for HPLC were as follows: Hilber prepacked column RT (10×250 mm) with Lichrosorb RP-18 (Merck, Darmstadt, Germany); water/acetonitrile/methanol/acetic acid (79.5:18:2:0.5, v/v/v/v) as the mobile phase; flow rate of 3 ml/min; UV-VIS spectrophotometric detector adjusted at 280 nm. Each eluted peak was collected, the solvent was evaporated under vacuum at 40 °C, and was finally stored at −20 °C until characterization by Proton (^{1}H) and Carbon (^{13}C) Nuclear Magnetic Resonance (NMR) Spectroscopy. The IGDE and MGDE solutions were endotoxin free. The possible endotoxins present in the IGDE and MGDE solutions were removed using Pierce Detoxi-Gel Endotoxin Removing Gel (Pierce Botechnology—Rockford, IL, USA).

2.3. Animals: mice and rats

The mice and rats used in this study were maintained under specific pathogen-free conditions. The animals were housed in laminar-flow cages maintained at a temperature of 22±2 °C and a relative humidity of 50–60%, under a 12:12 h light–dark cycle. The animals were kept under these conditions for at least one week before the experiment.

Male BALB/c and C57BL/6 mice, 6–8 weeks old, were matched for body weight (18–22 g). The male Sprague–Dawley rats, weighing 270–300 g, were also matched for body weight before use. Animal experiments were performed in accordance with ethical guidelines of Helsinki Declaration (1975), the Institutional Animal Care and use of UNICAMP and UFAM universities.

2.4. Mouse tumor models

2.4.1. Non-syngeneic ascites tumor model

Ehrlich ascites tumor was maintained in male BALB/c mice by serial transplantation. Tumor cell suspensions were prepared in a balanced salt solution at pH 7.4 at final concentrations of 6×10^{7} viable cells/ml. Mice were inoculated intraperitoneally (i.p.), on day 0, with 6×10^{6} viable tumor cells per BALB/c mouse in a volume of 0.1 ml. Viability, assessed by Trypan blue dye exclusion method, was always found to be 95% or more (Justo et al., 2000; Da Silva et al., 2007).

2.4.2. Syngeneic solid tumor model

Lewis lung carcinoma cells were kept in DMFE-12 medium (Hyclone Laboratories) supplemented with 10% foetal calf serum (Gibco, USA), 100 U/ml penicillin, 100 μg/ml streptomycin and 0.5 μg/ml amphotericin B 10. In the syngeneic model, on day 0, viable cells (2×10^{5}