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Pharmacochemistry

Effects of Poly[3-(3,4-dihydroxyphenyl)glyceric acid] on the Inflammatory Response of Tumor-Activated Hepatic Sinusoidal Endothelium

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(Presented by Academy Member E. Kemertelidze)

ABSTRACT. The effects of poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDGA) from *Symphytum asperum* on primary cultured hepatic sinusoidal endothelium (HSE) and its adherence to cancer cells were evaluated. The compound significantly induced TNF-α production from normal and tumor-activated cells, supporting its potential as immune defense modulator. Besides, PDGA completely abrogated the adhesion of murine B16 melanoma cells to tumor-activated HSE, without any detectable effect on basal condition-cultured HSE. Consistent with these anti-adhesive effects, PDGA also prevented melanoma cell adherence to recombinant vascular endothelial growth factor (VEGF)-treated HSE. *© 2008 Bull. Georg. Natl. Acad. Sci.*

Key words: *Symphytum asperum, poly[3-(3,4-dihydroxyphenyl)glyceric acid], hepatic sinusoidal endothelium (HSE), vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-a), B16 melanoma (B16M), B16M-conditioned medium (B16M-CM).*

Introduction

Caffeic acid and its derivatives of natural and synthetic origin have antioxidant, anti-inflammatory, hepatoprotective, antimutagenic, anticancer, immunomodulatory, pro-apoptotic activity and inhibitory effect on angiogenesis, tumor invasion, and metastasis. Their radical-scavenging and antioxidative activities are mainly due to the presence of two phenolic hydroxy groups at *ortho* positions [1].

It is suggested that antitumor activity of caffeic acid and its derivatives is related to the immunomodulatory properties of the compounds and their capacity to induce apoptosis and necrosis [2].

Cancer cells are dangerous not so much because they have lost the brakes on their growth. Rather, it is their ability to metastasize, escape from the original tumors and spread in the body, that makes cancer so tenacious and deadly. Last researches in the cell and molecular biology of inflammation, hypoxia and oxidative stress showed that these factors may have a critical role in processes involved in cancer metastasis, such as cancer cell adhesion to capillary endothelium, elimination of genetic controls on the growth and death of circulating cancer cells and angiogenesis [3,4]. The adhesion of cancer cells to the endothelial lining of blood vessels, which is important for metastasis, is promoted by the action of

IL-1, TNF-α and other cytokines [5,6].

Cancer cell adhesion to the endothelium is a key regulatory step of prometastatic effects of inflammation. Not surprisingly, cancer cells frequently use the same molecular tools (adhesion molecules, cytokines, chemokines, chemokine receptors) and pathways as leukocytes do to colonize at distant anatomical sites during inflammation. Exogenous proinflammatory cytokines can promote cancer cell adhesion to endothelium and metastasis. Elevated concentrations of proinflammatory cytokines IL-1α, TNF-α, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF) and IL-8 have been detected in the supernatant of human cancer cell lines. On the other hand proinflammatory cytokine blockade inhibits cancer metastasis development under experimental conditions, suggesting that proinflammatory mediators regulate host-tumor cell interactions contributing to arrest and implantation of disseminated cancer cells in target organs [7].

Angiogenesis, or formation of new blood vessels from pre-existing ones, is essential for normal development and wound healing/reproductive functions. Abnormal regulation of angiogenesis has been implicated in the pathogenesis of several disorders, including cancer. Angiogenesis is a crucial process in tumor progression. Among many tumor derived angiogenic factors that directly stimulate the endothelial motility and proliferation, VEGF is the most potent peptide that acts as specific mitogen for vascular endothelial cells. VEGF is a potential stimulator of angiogenesis because its binding to appropriate receptors has been shown to promote endothelial cell migration and proliferation, two key features required for the development of new blood vessels. In addition, VEGF increases vascular permeability, which may also contribute to angiogenesis and tumor growth. Recognition of the central role of VEGF in angiogenesis has led to the hypothesis that its inhibition may represent a novel and effective approach to the treatment of cancer and other conditions characterized by pathologic angiogenesis [8]. One of the most promising strategies for treating cancer is the addition of anti-angiogenic therapy to therapeutic regimens. Angiogenesis is essential both for the growth of a primary tumor and for successful metastasis. As a result of intense research in this field, a number of antiangiogenic agents have been identified and have demonstrated with varying degrees of success in inhibiting the growth of solid tumors and metastasis [9]. Several naturally occurring polyphenolic antioxidants exhibit anticancer effects due to inhibition of VEGF secretion by cancer cells, and growth inhibition and apoptosis in these cells [10,11].

Recently we have reported about the isolation of the first representative of a new class of natural polyethers - regular caffeic acid-derived polyether,

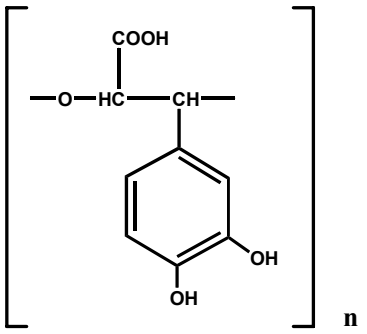

Fig. 1. Poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)glyceric acid] (PDGA) from *S. asperum* and *S. caucasicum*.

namely, poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl) glyceric acid] or poly[3-(3,4-dihydroxyphenyl)glyceric acid] **(PDGA)** (Fig.1) from Caucasian species of comfrey - *Symphytum asperum* and *S. caucasicum* and its immunomodulatory (anticomplementary) and antioxidative activities [12-17].

The goal of the present work was the evaluation of the effects of **PDGA** from the roots of *S.asperum* on primary cultured hepatic sinusoidal endothelium (HSE) and its adherence to cancer cells, because the liver is one of the most metastasized organs of the human body. For the realization of this aim we used a model of B16 melanoma (B16M) cell adhesion assays in B16M-conditioned medium (B16M-CM)-treated and VEGF-treated HSE cells *in vitro*.

Materials and Methods

Isolation of PDGA from the roots of *S.asperum.* Plant raw material - *S.asperum* roots were collected in mountain regions of Georgia. Voucher samples are deposited in the herbarium of the Kutateladze Institute of Pharmacochemistry (Tbilisi). The polymer was obtained by hot water extraction of the air-dried plant material [18] with subsequent ultrafiltration on membrane filter with cut-off value of 1000 kDa using Amicon Ultrafiltration System [2] (yield 2.75% based on dry roots).

B16 Melanoma (B16M) cell adhesion assay to primary cultured hepatic sinusoidal endothelial (HSE) cells. B16M cells were labeled with 10 μg/ml 2',7'-bis-(2-carboxyethyl)-5,6-carboxyfluorescein-acetoxymethylester (BCECF-AM) solution. This non-fluorescent sterase substrate BCECF-AM is accumulated by tumor cells and hydrolyzed to the fluorescent product BCECF which becomes trapped inside the live cells. After gently washing, $2x10^5$ cells/well were added to 24-well plate primary cultured HSE cells and 8 minutes later, the wells were washed three times with fresh medium. Cell adherence was calculated from absorbance at 485 nm using a fluorometric microplate reader (Multiskan Ascent, Thermo Labsystems). The number of adhered cells (registered in fluorescence arbitrary units) was expressed as percentage of the initial number of cells, and calculated for each well as follows: Fluorescence after well washing/(Fluorescence before washing – non-specific fluorescence before tumor cell addition)

Quantification of TNF-α. Release of TNF-α from primary cultured HSE cells was measured using the ELISA kit from R&D Systems based on anti-mouse TNF-α monoclonal antibody, as suggested by the manufacturer (R&D Systems, Minneapolis, MN).

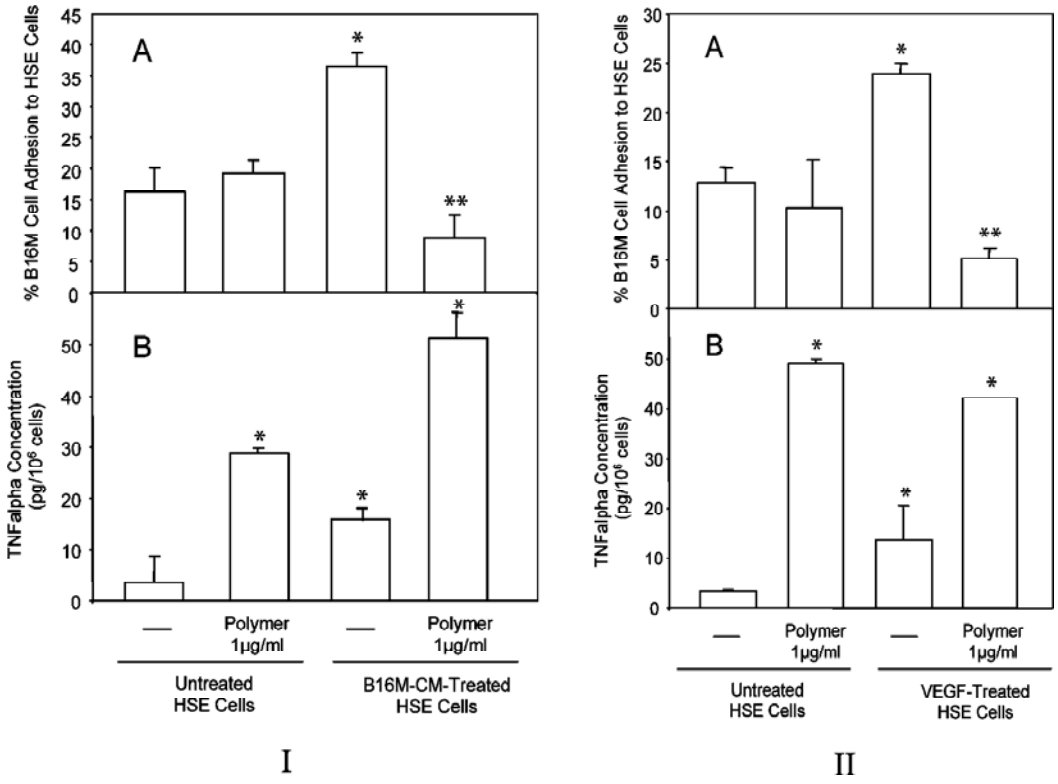

Fig. 2. Effects of PDGA on TNF-α secretion and B16M cell adhesion in B16M-conditioned medium (B16M-CM)-treated (I) or VEGF-treated (II) HSE cells *in vitro*.

Cultured HSE cells were incubated in the presence or absence of B16M-CM or 10 ng/ml murine VEGF for 8 hours. In some experiments, both untreated and treated HSE cells received 1 μg/ml **PDGA**, 30 minutes before B16M-CM or VEGF **(A)**. Cell adhesion assays were performed as described in Methods. **(B)** HSE supernatants were removed before cell adhesion and TNF-α concentration was measured by ELISA. Data represent the mean ±SD of 2 separate experiments performed using 2 different preparations of HSE cells, each in 3 replicates (n=6). Differences in the percent of adhering cells and TNF-α production versus untreated HSE cells (*) and versus B16M-CM or VEGF-treated HSE (**) were statistically significant ($P<.001$) by ANOVA and Bonferroni´s post-*hoc* test.

Results and Discussion

The **PDGA** was obtained as described in Materials and Methods and its identity was confirmed according to UV, IR and NMR spectral data [15-17].

The inflammatory response of tumor-activated endothelial cells has been chosen as a target-oriented strategy for the **PDGA** with potential inhibitory effect on the mechanism of circulating cancer cell implantation in HSE. The procedure for this biological testing of anti-metastatic activity involves the following sequence of studies *in vitro:* determination of **PDGA** inhibitory effects on proinflammatory cytokine release, and subsequent adhesiveness for cancer cells, of endothelial cells treated with conditioned media from cancer cell cultures.

The **PDGA** significantly induced TNF-α production from normal and tumor-activated HSE cells, supporting its potential as immune defense modulator. Besides, the polymer completely abrogated the adhesion of murine B16 melanoma cells to tumor-activated HSE, without any detectable effect on basal condition-cultured HSE. Consistent with these anti-adhesive effects, the **PDGA** also prevented melanoma cell adherence to recombinant VEGF-treated HSE (Fig. 2). Anti-adhesive effects occurred in the presence of high TNF-α levels, suggesting that the compound blocked down-stream molecular mediators of tumor factor- and VEGF-induced adhesion [6].

ფარმაკოქიმია

პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავას] მოქმედება სიმსივნით გააქტივებული ღვიძლის სინუსოიდური ენდოთელიუმის ანთებით პასუხზე

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შესწავლილია *Symphytum asperum*-ის პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავას] მოქმედება სიმსივნით გააქტივებულ ღვიძლის სინუსოიდურ ენდოთელიუმზე (HSE) და მასზე სიმსივნის უჯრედების ადჰეზიის უნარი. ეს ნივთიერება იწვევს, როგორც ნორმალური, ისე სიმსივნით გააქტივებული უჯრედების მიერ სიმსივნის ნეკროზის ფაქტორის (TNF-α) გენერაციის მნიშვნელოვან პროდუცირებას, რაც მიუთითებს მისი, როგოც იმუნური სისტემის მოდულატორის პოტენციალზე. გარდა ამისა, პოლიმერი ახდენს სიმსივნით გააქტივებულ HSE-ზე თაგვის B16 მელანომას უჯრედების ადჰეზიის სრულ ბლოკირებას. ნივთიერება ახდენს აგრეთვე რეკომბინანტური ვასკულარული ენდოთელიური ზრდის ფაქტორით (VEGF) დამუშავებული HSE-ზე მელანომას უჯრედების ადჰეზიის პრევენციას.

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