Journal of Traditional and Complementary Medicine 6 (2016) 219–223

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Original article

Effect of *Fagonia arabica* on thrombin induced release of t-PA and complex of PAI-1 tPA in cultured HUVE cells

Prutha D. Aloni [a], Amit R. Nayak [a], Sweta R. Chaurasia [a], Jayant Y. Deopujari [a], Chhaya Chourasia [c], Hemant J. Purohit [b], Girdhar M. Taori [a], Hatim F. Daginawala [a], Rajpal S. Kashyap [a, *]

[a] *Biochemistry Research Laboratory, Central India Institute of Medical Sciences, Nagpur 440010, Maharashtra, India*
[b] *Environmental Genomics Unit, NEERI, Nehru Marg, Nagpur 440020, Maharashtra, India*
[c] *Matru Sewa Sangh, Maternity Home, Kothi Road, Mahal, Nagpur 440002, Maharashtra, India*

ARTICLE INFO

Article history:
Received 24 December 2014
Received in revised form
10 February 2015
Accepted 4 March 2015
Available online 3 April 2015

Keywords:
HUVE cell line
Fagonia arabica (*FA*)
Thrombin
Tissue plasminogen activator (t-PA)
Plasminogen activator inhibitor-1 (PAI-1)

ABSTRACT

Fagonia arabica (*FA*) possesses a thrombolytic property which has been earlier reported in our laboratory. Current study was undertaken to investigate the effect of aqueous extract of *FA on* thrombin-induced tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) release from cultured human umbilical vein endothelial cell line (HUVE) for studying its clot lytic activity. For this, establishment of cell line model has been done by isolating the cells from human umbilical cord. Cell toxicity was evaluated using XTT assay. Estimation of t-PA and PAI-1 t-PA complex were done using ELISA technique. Thrombin treatment induces the t-PA and PAI-1 release from HUVE cell line, and *FA* treatment was found to antagonize the thrombin induced t-PA and PAI-1 release. Our preliminary results suggest that *FA* may be used as an alternative to thrombolytic drug. However, study demands further experiments using animal model of thrombosis to establish the role of *FA* as a novel thrombolytic drug.
Copyright © 2015, Center for Food and Biomolecules, National Taiwan University. Production and hosting by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Hemostasis is the finely tuned balance between two systems i.e., coagulant and fibrinolytic system. Failure of hemostasis results in the formation of blood clot in the circulatory system.[1] According to Virchow's triad, the three broad categories of factors that contribute to thrombosis are – hypercoagulability, hemodynamic changes (stasis, turbulence) and endothelial injury/dysfunction.[2]

Thrombolytic drugs such as tissue plasminogen activator (t-PA), alteplase, recombinant tissue plasminogen activator (rc-tPA) etc. are used all over the world for the treatment of thrombotic diseases but are associated with high therapeutic cost.[3–7] On the contrary streptokinase and urokinase are cost effective,[8,9] but suffer from limitations of serious bleeding complications along with reocclusion and reinfarction in some cases.[10]

The plant kingdom represents a vast reservoir of important medicinal properties (phytochemicals) which are useful for curing various diseases.[11–13] In recent years, Indian research is focusing on evaluation of specific traditional ayurvedic medicine for treatment of disorders for which it has been prescribed for a long time in order to find its active component and also to understand the mechanism of action.[2, 6–7, 14–15] Therefore, use of herbal drugs with antiplatelet, antithrombotic and thrombolytic properties can be used as a cost effective alternative for the treatment of thrombotic diseases.[16–22]

Earlier it has been reported that herbs such as *FA* have thrombolytic properties and may be used as substitutes for available thrombolytic drugs.[7,14] However, mechanism of action by which *FA* induces the clot lysis is required to be evaluated.

Endothelial cell line has been widely used to study various biomarkers associated with thrombotic diseases. Endothelial cells play an important role in coagulant as well as fibrinolytic system by secreting various factors such as cytokines, vasoregulatory substances, adhesion molecules, growth factors, factors related to coagulant and fibrinolytic factors etc.[23]

* Corresponding author. Biochemistry Research Laboratory, Central India Institute of Medical Sciences, Nagpur 10, Maharashtra, India. Tel.: +91 712 2236441; fax: +91 712 2236416.
E-mail address: raj_ciims@rediffmail.com (R.S. Kashyap).
Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2015.03.002
2225-4110/Copyright © 2015, Center for Food and Biomolecules, National Taiwan University. Production and hosting by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

In the regulation of hemostasis, thrombin is a central enzyme having essential role in coagulant as well as fibrinolytic system. Thrombin is directly responsible for releasing tPA from endothelial cell line which favors fibrinolysis by converting plasminogen to plasmin. Thrombin also releases PAI-1 which inhibits the activity of tPA and favors coagulation.[23] Therefore, keeping the above facts in mind, we have planned to investigate the effect of aqueous extract of *FA* on thrombin-induced t-PA and PAI-1 tPA complex release in cultured HUVE cell line to assay the clot lytic activity.

2. Materials & methods

2.1. Materials

The L-15 Medium (Leibovitz's), Medium 199**,** CollagenaseType I, Gelatin solution (2%), Antibiotic-Antimycotic solution (100X), HiEndo ^{XL} Endothelial Cell Expansion medium (Reduced serum), Heparin sodium salt, Trypan Blue, Trypsin solution (0.25%) were procured from Himedia. Fetal Bovine Serum (FBS), Poly-L-Lysine, Thrombin from Bovine Plasma and 2, 3 bis (2-methoxy- 4 nitro 5-sulfophenyl) 2H- tetrazolium- 5 carboxanilide inner salt (XTT) were purchased from Sigma (St. Louis, MO,USA). Other chemicals are of analytic grade. Cell culture plastic wares were purchased from Axiva Sichem Pvt. Ltd. (New Delhi, India).

2.2. Plant material

The plant *FA* (common name: Dhamasa, belongs to the family: *Zygophyllaceae*) was purchased and identified by Dr A. M. Mujumdar, Agharkar Research Institute Pune, Maharashtra, and the authentication number is Auth07-89.

2.3. Herbal preparation

Dried Herbs, *FA* was purchased from local market and was verified with the help of a Botanist and an Ayurvedic Physician. Water extract was prepared using Soxhlet extractor. The extract was prepared only once and used in all experimental set up.100 mg extract was suspended in 10 mL distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.2- micron syringe filter. This filtrate obtained was used as a stock solution (10 mg/ml).

2.4. Isolation and primary culturing of HUVE cell line from human umbilical cord

All methods for the experiments included in this study were approved by Ethical Committee of Central India Institute of Medical Sciences, Nagpur. Endothelial cells were obtained from human umbilical cord vein by the method of Jaffe et.al. & Ulrich-Merzenich et.al. under aseptic conditions.[24,25] Briefly, the cord was severed from the placenta soon after birth, placed in a sterile container filled with the transport medium i.e., L-15 medium (10%FBS & 10X Antibiotic-Antimycotic solution). Before isolation the cord was carefully inspected, and all areas with clamp marks were cut off. The umbilical cords were dipped in betadine solution in the Phosphate Buffer Saline (PBS) (1:5) for 2 min followed by 70% ethanol for 1 min. Blood and blood clots from the umbilical cord were removed and fresh cuts were made on both the ends. The vein (largest opening in the cord) was perfused with 5–10 mL of cold PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4.7H_2O$, 1.4 mM KH_2PO_4, pH 7.4) for five-six times to wash out the blood and blood clots. Two to three millilitres of collagenase solution (1 mg/mL) were injected by the syringe (2 mL) through the needle (top winged infusion set) with the plastic needle sheath ON, into the vein and then the needle was clamped in place with artery forceps. The other end of the umbilical cord was also clamped with an artery forcep. The umbilical cord was placed in the beaker containing PBS and incubated at 37 °C.After incubation, the collagenase solution containing the endothelial cells was flushed from the cord vein by perfusion with medium 199 (containing 10%FBS). The effluent was collected in the 15 mL centrifuge tube and was centrifuged for 10 min at 1000 rpm. Endothelial cells were cultured in the HiEndo^{XL} Endothelial Expansion Medium with 10x antibiotic-antimycotic solution and seeded in the 1% gelatin or poly –L-lysine coated T-25 flask. Cultures were identified as HUVE cell line through their cobblestone/polygonal morphology under the light inverted microscope and by the presence of von Willebrand factor (vWF).

The confluent monolayer was obtained after 7–8 days. At confluence, cells were harvested by treatment with 0.05% trypsin-0.02% EDTA, the trypsin was inactivated by the addition of medium 199 containing 10% FBS and the cells were routinely passaged at a constant 1:3 split ratio. Between three – five passages of HUVE cells were used for experiment.

2.5. Characterization of HUVE cell line from human umbilical cord

Characterization of HUVE cell line was done by estimating vWF in the conditioned medium of HUVE cells in comparison with conditioned medium of Peripheral Blood Mononuclear cell line (PBMC) as a control since they do not express vWF, while HUVE cells are reported to express and secrete considerable amount of vWF.[26–29] In brief, 100 μl of conditioned medium of sample (HUVE cell line) and control (PBMC cell line) was added to the microtitre wells and the plate was incubated at 37 °C for 90 min and thereafter blocked with blocking buffer (0.5% BSA in PBS-T). Incubation was carried out at 37 °C for 45 min. The primary antibody (vWF antibody, 1:100) raised in goat was added in the microtitre wells and the plate was incubated at 37 °C for 2 h. Then, secondary antibody (Rabbit anti-goat IgG-HRP, 1:1000) was added to the wells and incubated for 1 h. 100 μl of TMB/H_2O_2 substrate was added to the wells and incubated at room temperature for about 5–8 min. The reaction was stopped with 100 μl of 1N H_2SO_4. The absorbance of color in each well was read at 450 nm. Each sample was tested in triplicate.

2.6. Analysis of cell toxicity by XTT assay

Cell toxicity was assayed using the XTT assay as described earlier.[30] For cell toxicity assay, 2×10^5 cells/well HUVE cells were incubated with different concentrations of *FA* i.e., 100 μg/ml, 500 μg/ml & 1000 μg/ml. Briefly, after 24 h of incubation, 20 μl of XTT containing phenazine metrosulphate (PNS) (0.92 mg/ml XTT solution) was added to each well. After a four –hour incubation period, the plate was read at wavelength of 450 nm on a Stat Fax 325 + microtitre plate reader (Ark Diagnostic, Mumbai, India). Results of XTT assay were expressed in terms of percentage viability considering the viability of untreated cells (control) as 100%.

2.7. In vitro thrombosis model

The *in vitro* thrombosis model was established by the method described by Zhao et. al.[31] Passages between 3–5 of HUVE cells were used for experiment. 2×10^5 cells/well HUVE cells were cultured in the 1% gelatin/poly –L- lysine coated 24-well tissue culture plate. The culture medium was discarded and next, fresh, medium with reduced serum was added. The plates were induced with 3 U/mL of thrombin with presence and absence of different concentration of *FA* (added 10 min after the application of

Download English Version:

https://daneshyari.com/en/article/3099670

Download Persian Version:

https://daneshyari.com/article/3099670

Daneshyari.com