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Research paper

Collegenase activity in fig latex could contribute to its efficacy in ethnomedicinal preparations

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ABSTRACT

Ficus carica, the common fig has been widely used for centuries for medicine as well as food. Fig latex is used for the treatments of cancer, inflammatory diseases, bacterial and gastrointestinal nematode infections, warts and skin diseases. The aim of the study was to investigate if some of the fig latex applications could be attributed to collagenase activity. The usage of figs in the Western Balkans indicated that the latex of unripe fruits is often used for treatments that could involve remodeling of connective tissue. Collagenase activity in fig latices collected in spring was twice as high as the one detected in summer. Collagenase improved diffusion of low-molecular-weight model molecule through the gelatin hydrogel. Fig latex collagenase was stable during boiling and in the simulated gastric conditions for up to 1 h. The presence of fig latex collagenase in traditional medicine preparations could increase treatment efficacy by hydrolyzing collagen present in extracellular matrix and facilitating the penetration of active molecules through the connective tissue.

1. Introduction

The fig tree (*Ficus carica*) has been cultivated in southern parts of temperate zones to be used as food and medicine. Fig latex is produced by laticiferous cells and it has been suggested that the latex secretion is a defense mechanism against wounding or pests such as insects and microorganisms (Oliveira et al., 2010). It has been extensively investigated for its proteolytic enzymes, LMW (low molecular weight) compounds and rubber (Kang et al., 2000). The use of fig fruit and latex for the treatment of cancer, inflammatory diseases, bacterial and gastrointestinal nematode infections, as well as warts and skin diseases, has been part of traditional medicine worldwide, especially in developing countries (Lansky et al., 2008).

Representing a rich source of proteolytic enzymes, many of the ethnopharmaceutical uses of fig latex has been traditionally attributed to ficin activity (Lansky and Paavilainen, 2011). Ficin is a common name for endoproteolytic activity in latex of the genus *Ficus*. Ficin (EC 3.4.22.3) is a proteolytic enzyme present in the latex of fig trees (*F. glabrata* and *F. carica* species) (Azarkan et al., 2011; Devaraj et al., 2008). Ficin forms are recognized as sulfhydryl enzymes which contain cysteine residue in their active site and have molecular weight of about 24 kDa.

The activities of fresh latex usually attributed to proteases (e.g. ficin) include treatment of various skin diseases involving itching and eruptions, as well as treatment of rheumatism and wart removal (Lansky et al., 2008). The usage of fig latex in wart removal is at least as effective as conventional cryotherapy (Bohlooli et al., 2007). It is proven, as well, that proteases derived from fig activate human factor X and affect haemostasis (Richter et al., 2002). Recent studies have shown that the effects of papaya, fig and pineapple latices or fruit extracts against rodent gastrointestinal nematodes are the consequences of the proteolytic activity leading to the complete digestion of nematodal cuticle (Stepek et al., 2007).

Furthermore, latex of *F. carica* is used in traditional medicine for the softening of solid tumors and debridement and healing of ulcers. Fig latex is usually administered combined with other botanical ingredients such as blue flag (*Iris versicolor* L.), barley, fenugreek, ginger and hot pepper. The most common route of administration has been external, but it is also given orally (boiled or fresh, mixed with flour, starch or dried milk) (Lansky et al., 2008).

A novel protease expressed in fig latex has been recently described (Raskovic et al., 2014). The protease belongs to serine protease family and cleaves preferentially collagen and gelatin. The enzyme has molecular weight of about 45 kDa. Additionally, this enzyme exhibits a very broad range of pH and temperature stability.

Since some of the ethnomedicinal activities of fig latex are clearly dependent of fig proteases (Lansky and Paavilainen, 2011),

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we postulated a hypothesis that collagenolytic fig latex protease, rather than ficin, could enable rearrangements of connective tissue and facilitate diffusion of active LMW (low molecular weight) molecules through the skin and internal connective tissues. The list of the use of fig latex in ancient and contemporary ethnomedicine that could have collagenase activity involved is presented in Table 1.

The use of fig latex in the Western Balkans as a folk medicine in several pathologies and conditions (such as skin diseases: hardening of skin, acne, psoriasis; scars and wart removal; treatment of ulcers and tumors), was investigated with a particular emphasis on its possible contribution to collagenolytic activity as a part of its molecular mechanism of action.

2. Material and methods

2.1. Materials

Collagen from rat tail; IAA; E-64 (*N*-[*N*-(L-3-*trans*-carboxyirane-2-carbonyl)-L-leucyl]-agmatine); Pefabloc SC; Pepstatin A; EDTA (ethylenediaminetetraacetic acid disodium salt dehydrate); sodium azide, bovine serum albumin; pepsin A (3.64 U/mg of protein); BPB (bromphenol blue) were purchased from Sigma–Aldrich (Steinheim, Germany). Gelatin was purchased from Merck (Darmstadt, Germany). Unstained protein molecular weight marker was bought from Thermo Scientific (Rockford, IL, USA). All other chemicals were commercial products of analytical grade and were used without further purification.

2.2. Ethnopharmacological survey

Ethnopharmacological survey was carried out in the period 2012–2014 in Adriatic cost-side Montenegro (Bar region) and central Serbia (Sumadija region). Seventeen subjects (8 in Montenegro and 9 in Serbia) who had practiced traditional medicine were enrolled in the survey. Written consent was obtained from every study subject prior to the survey after which they were asked for their knowledge of the usage of figs (fruits, milk and leaves) in the treatment of any health condition. The specific questions included the part of the plant used; medicinal uses; and route of administration.

2.3. Latex collection

Fresh latex was collected by mechanical incision every 15 days in the period May–August 2013 from the fig trees (*F. carica* var. Brown Turkey) in Bar, Montenegro. Each time, 10 mL of the latex fluid was collected from the same fig trees (three in total, each time approximately 10 fruits were used). The latex was immediately stored at −20 °C until used.

2.4. Protein concentration determination

The protein concentration was quantified by the dye binding method (Bradford, 1976) using bovine serum albumin as a standard.

2.5. Proteolytic assay

Proteolytic activity of latex samples was estimated using BAPNA as substrate. BAPNA hydrolyzing activity was quantified according to Raskovic et al. (2014). The assay was run in triplicate. Proteolytic activity was calculated as the amount of enzyme that catalyzed the release of 1 μM of product per minute under standard conditions (International Unit of enzyme activity—1IU). The specific activity was calculated as the ratio of the enzymatic activity to the total protein content of the sample, and expressed in mU/mg.

2.6. Collagenolytic activity

Collagenolytic activity of latex samples was assayed as previously described (Raskovic et al., 2014). One unit of collagen digestion activity (CDU) was defined as the amount of enzyme that releases peptides from collagen equivalent in ninhydrin colour to 1 mmol of leucine in 5 h.

The specific activity was calculated as the ratio of the enzymatic activity to the total protein content of the sample, and expressed in CDU/mg. The assay was run in triplicate.

2.7. Diffusion assay

In order to check if fig latex collagenases could facilitate diffusion of LMW molecules through the extracellular matrix, the authors applied a modified protocol, the same one as used for determining migration of cells through the collagen hydrogel (Shin et al., 2012). The experiment was conducted in glass capillary tubes (2 mm inner diameter, 5 cm high). Fig latex (sample collected on May 1st 2013) was added to 1% gelatin solution in PBS buffer and specific inhibitors were added. Final concentration of latex proteins was 0.5 mg/mL. Final concentrations of inhibitors were: 2 mM each: IAA; Pefabloc SC and EDTA; 2 μM E-64 and 1.46 μM Pepstatin A. Controls were set by adding PBS instead of latex or inhibitors. Gelatin hydrolysis was allowed to proceed for 30 min at room temperature. The tubes were chilled at 10 °C to allow gelatin to form hydrogel for 15 min. After formation of hydrogel, 0.05 mL of 10 μg/mL BPB solution in PBS was carefully added to the top of the

Table 1
The use of fig latex in ancient and contemporary ethnomedicine that could involve collagenase activity.

Use	Medicament	Place	Route of administration	References
Scar removal	Latex	Persia	External application	(Lansky and Paavilainen, 2011)
Warts	Fresh latex	Iran	External application	(Bohlooli et al., 2007)
	Latex	Italy	External application	(De Feo et al., 1992)
	Fresh latex	Turkey	External application	(Yeşilada et al., 1995)
Liver cirrhosis	Latex (collected in the beginning of spring)	Syria, Persia, Turkey, England	Poultice, drink	(Lansky et al., 2008)
Head wounds	Latex (collected in the beginning of spring)	Turkey	Ointment	(Lansky et al., 2008)
Softening of solid tumors	Boiled latex, mixed with other ingredients or fresh latex only (collected in the beginning of spring)	Syria, Persia, Turkey	Poultice, drink often together	(Lansky et al., 2008)
Debridement and healing of ulcers	Latex with egg yolk or vegetable oil	Germany, Italy	Poultice, liniment	(Lansky et al., 2008)