ANTI-INFLAMMATORY PROPERTIES OF NATURALLY OCCURRING BIOTECHNOLOGICALLY PRODUCED PHENYLPROPANOID GLYCOSIDES

L.G. KORKINA [1,2], S. PASTORE [1] AND R. DAL TOSO[3]

[1] Laboratory of Tissue Engineering & Skin Pathophysiology, Istituto Dermopatico dell'Immacolata (IDI IRCCS), Rome, Italy,

[2] Department of Molecular Biology, Russian State Medical University, Moscow, Russia,

[3] Institute of Biotechnological Research (IRB), Vicenza, Italy

INTRODUCTION

Phenylpropanoid glycosides (PPGs, also synonymous of phenylethanoid glycosides) are water soluble derivatives of phenylpropanoids (PPs), the largest group of natural polyphenols widely distributed in the plant kingdom. At present, more than two hundred different PPGs are known, extracted from aerial parts and roots of the plants belonging to the families of Labiateae, Asteraceae, Oleaceae, Liliaceae, and others. Both verbascoside (VB) and teupolioside (TP) belong to the PPGs group and are structurally characterized by caffeic acid (phenylpropanoid moiety) and 4,5 hydroxyphenylethanol (phenylethanoid moiety) bound to a β-(D)- glucopyranoside trough an ester and glycosidic links, respectively. In the VB molecule, rhamnose is linked in sequence to the glucose molecule. The monocarbohydrate chain of TP consists of three moieties, rhamnose, galactose, and glucose. Chemical structure of verbascoside There is a growing evidence that PPGs, like other plant polyphenols in general and PPs in particular, are powerful antioxidants either by direct scavenging of reactive oxygen and nitrogen species, or by acting as chain-breaking peroxyl radical scavengers. Polyphenols such as PPGs and bioflavonoids with two adjacent –OH groups, or other chelating structures, can also bind transition metals, first of all iron and copper, in forms poorly active in promoting free radical chain reactions. Recently, PPGs have been reported to possess multiple beneficial effects for human health. Indeed, they have been effective in the chemoprevention of tumors; some have anti-inflammatory activity, while others have anti- thrombotic, wound healing, and cardio-protective actions. These health effects of PPGs have been traditionally explained in terms of the prevention of free radical-associated and transition metal-mediated cell and tissue damage. Natural accelerators of wound healing with anti-inflammatory action are of great interest for surgery, dermatology and modern cosmetology. For example, in the course of T cell-driven skin inflammatory diseases, activated Th1 lymphocytes infiltrating the dermis and the epidermis are the major source of potent proinflammatory cytokines TNF-α and IFN-χ. These cytokines initiate a program of increased keratinocyte expression of inflammatory mediators, including adhesion molecules, growth factors such as GCSF, cytokines and chemokines. In particular, prominent expression of CCL2 (Monocyte chemoattractant protein 1, MCP-1), CCL5 (RANTES), CXCL8 (interleukin 8, IL-8) and CXCL10 (IFN-χ-induced protein of 10 kDa, IP-10) in keratinocytes is a common feature of T cell-mediated skin inflammation, and mediates the recruitment of Tcells, granulocytes, dendritic cells, and monocytes in the skin. The recruitment of activated granulocytes and monocytes leads to overproduction of reactive oxygen and nitrogen species (ROS and RNS, respectively). The prolonged overproduction of these reactive species can cause severe tissue damage, impair wound healing, and result in DNA mutations that can lead to tumorigenesis. The endogenous protective mechanisms include induction of antioxidant and detoxifying phase II enzymes in the skin cells (keratinocytes and fibroblasts) and migrated inflammatory leukocytes (granulocytes and monocytes). Two ROS-inducible enzymes, glutathione-1 Stransferase (GST) and heme oxygenase-1 (HO-1) are thought to be of extreme importance in the cytoprotection during cutaneous wound repair. Naturally occurring PPGs seem to be excellent candidates to promote skin regeneration and ameliorate skin inflammation due to their ROS scavenging, antioxidant, iron chelating, and GST inducing properties. However, the industrial development and utilization of PPGs for medicinal use are limited because their chemical synthesis is extremely complex and expensive. Their extraction from mature plants has a very low yield, the final PPGs-containing products are poorly standardized due to unavoidable variations in the plant growth conditions, and they are often contaminated with environmental pollutants. There is now growing interest in the biotechnological approach to produce plant-derived active substances using non-genetically modified plant cell cultures. Plant cell cultures derived from medicinal plants are perfect sources of PPGs, biosynthesis of which could be specifically induced and directed to a certain compound depending on the nature of plant cells and a stimulus used.

MATERIALS AND METHODS

Plant cell cultures and PPGs isolation and analyses

The extracts containing VB (97%, 56% w/w) and verbascoside-free (<1%) samples were obtained from *Syringa vulgaris* plant cell line. Both purified TP (95% w/w) and raw extract containing 70% w/w TP were obtained from *Ajuga reptans* plant cell line. Both *Syringa vulgaris* and *Ajuga reptans* have been recognized as European ethnobotanical medicinal herbs effective in accelerating of wound healing, as anti-inflammatory, antirheumatic, anti-pyretic and anti-fungal remedies since ages. The stabilized and highly selected cell lines specified on the synthesis either VB or TP were obtained from sterilized dissected young *Ajuga reptans* leaves and *Syringa vulgaris* flowers, respectively. Cell cultures obtained in the ndustrial amounts were collected, homogenized, separated by centrifugation, and the solid residue discarded. The PPGs in the supernatant were recovered by solid phase extraction on XAD4 resin, followed by elution with an 80/20 ethanol/water (v/v) mixture. The further purification was performed by repeated column chromatography on C18 silica gel and Sephadex LH20 and subsequent crystallization. The raw extracts of TP (70%) and VB

(56%) contained other caffeic acid derivatives (approx. 10%) as was revealed by a chromatography-mass spectrometry analysis.

Keratinocyte cultures and analyses of pro-inflammatory mediators.

Epidermal sheets for keratinocyte cultures were obtained from healthy individuals undergoing plastic surgery (n= 4, two females and two males; age 25-45 y). Primary cultures were established as described. Keratinocytes were sub-cultured in the serum-free medium Keratinocyte Growth Medium™ (KGM; Cambrex, San Diego, CA, USA), prepared from the essential solution supplemented with 10 ng/ml epidermal growth factor, 0.4 μg/ml hydrocortisone, 5 μg/ml insulin, 2 ml bovine pituitary extract and antibiotics. Second- or third-passage keratinocytes were used in all experiments. In the 24 h preceding the experiments, 80% confluent keratinocyte cultures were switched to hydrocortisone-depleted medium.

Then, cells were treated with 100 ng/ml TNF-α and/or 100 U/ml IFN-γ for 24 h. Recombinant human TNF-α and IFN-γ were from R&D Systems (Abingdon, United Kingdom).

Pro-inflammatory mediators were measured in the cell supernatants by ELISA using correspondent kits from BD Biosciences (San Diego, CA, USA). All experiments were repeated with keratinocytes derived from at least three different donors.

RESULTS

The experiments with primary cultures of human keratinocytes activated by pro-inflammatory cytokines, TNT alpha and interferon-gamma taken alone or in combination, showed that the expression of chemokines for monocytes (MCP-1), for granulocytes and monocytes (IL-8), for dendritic cells (IP-10), growth factor GCSF **was effectively inhibited by both 97% VB and TP extracts** within micromolar range of concentrations (1-50μM). Both extracts were **more effective** inhibitors than **hydrocortisone and triamcinolone**, two "classical" anti-inflammatory agents. Taken together, our data allowed us to suggest that biotechnologically produced VB and TP extracts **exhibited remarkable antiinflammatory activity**.

Keratinocytes, being triggered by proinflammatory cytokines, produce growth factors and chemoattractants for pro-inflammatory cells such as granulocytes, monocytes, and T lymphocytes. Inhibition of pro-inflammatory cell migration to damaged skin would result in the moderate inflammatory response. The concentrated PPGs, being strong free radical scavengers and antioxidants, induce GST, the major phase 2 detoxifying enzyme, which could be of greater importance for tumor chemoprevention than for wound healing action 2

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