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Time-dependent bioactivity of preparations from cactus pear (*Opuntia ficus indica*) and ice plant (*Mesembryanthemum crystallinum*) on human skin fibroblasts and keratinocytes

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A B S T R A C T

Ethnopharmacological relevance: Traditionally and nowadays preparations from two xerophytic plants, the ice plant and cactus pear are used in dermatologic and cosmetic preparations. In spite of their daily use, little is known concerning the bioactivity of such extracts on skin cells. The purpose of this study was to investigate the effect of pressed juices from ice plant (McP) and two cactus pear polysaccharides (cold water soluble, NwPS; non swelling pectin, NPec) on the cell physiology of normal human dermal fibroblasts (NHDF) and HaCaT-keratinocytes due to composition, concentration and incubation time.
Materials and methods: Cactus pear polysaccharides were analyzed by high performance anion exchange chromatography with pulsed amperometric detection after hydrolysis with trifluoroacetic acid. Ice plant pressed juices were filtrated through a 1.2 μm (McPI) and 0.2 μm filter (McPII). Cell proliferation was measured with BrdU incorporation assay. Reduction of tetrazolium salts was applied to determine the metabolic activity (MTT) while necrotic effects were assessed by LDH-release measurements.
Results: Cactus pear polysaccharides differed predominantly in their glucose and uronic acid content. The filtration of pressed juices altered the amounts of high molecular weight compounds. The proliferation of NHDF and HaCaTs was significantly stimulated by cactus pear polysaccharides and ice plant pressed juices not until 72 h of incubation. McPI significantly increased the proliferation of NHDF and HaCaTs while significant effect of McPII was only observed in case of HaCaT-keratinocytes. A dependence on concentration was not observed. Metabolic activity was neither influenced by McPI nor by McPII independent of incubation time. The HaCaT proliferation was not significantly influenced by low concentrations of cactus pear polysaccharides however it was inhibited by 100 μg/mL NPec. 100 μg/mL of NwPS and 1 μg/mL NPec stimulated the proliferation of fibroblasts. The metabolic activity of NHDF was not affected neither by NPec nor by NwPS. Independent of the used concentration NwPS significantly enhanced the metabolic activity of HaCaTs after 48 h of incubation.
Conclusions: Pressed juices of common ice plant and polysaccharides of cactus pear influenced the cell physiology of human keratinocytes and fibroblasts predominantly in a time-dependent manner. The effect was also be related to the concentration and composition as well as the investigated cell type.
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1. Introduction

Plant extracts are widely used for dermatologic and cosmetic preparations (Reuter et al., 2010). Active extracts are usually directly applied after pressing (*e.g. Aloe vera*) or prepared by aqueous or ethanolic/aqueous extraction (Casetti et al., 2011). Traditionally such extracts are used for wound healing and skin regeneration. Nowadays the traditional usage is replaced by cosmetic and dermopharmaceutical applications. Common ice plant and cactus pear are the two model examples representing this shift from traditional to modern use.

Common ice plant (*Mesembryanthemum crystallinum* L., Aizoaceae) is a facultative halophyte, adapted to extreme environmental conditions by synthesis of protective substances (osmolytes) and antioxidant molecules such as betacyanins, mesembryanthin and other flavonoids (Hanen et al., 2009; Vogt et al., 1999). Further hydrophilic compounds were identified as sugar alcohols, organic acids, mono-, di- and polysaccharides (M'sakni et al., 2005; Wende et al., 2006). Nowadays the antioxidant potential of phenolic ingredients and the moisturizing effects are two well-appreciated properties of cosmetic formulations (Wende et al., 2006). Cactus pear (*Opuntia ficus-indica* L., Cactaceae) is a famous food plant in

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Mexico, Italy, Spain, Brazil, Chile, Argentina and California. *Opuntia* species were used traditionally for different diseases by native inhabitants of Mexico and Peru (Barradas, 1957; Martinez, 1959). In view of its relevance for nutrition, medicinal and cosmetical use, *Opuntia* species have been investigated considerably well with respect to its composition and bioactivities. Especially the mucilage was the focus of different scientific groups from Mexico and Chile. Though the bioactivity of cactus pear extracts has been widely investigated, only the hypoglycemic, antidiabetic (Stintzing and Carle, 2005) and wound healing promoting effect on rats (Trombetta et al., 2006) were attributed to the polysaccharides.

While both plants are associated with a beneficial effect on human skin (Martinez, 1959), little is known concerning the underlying principle and the responsible extract compounds. For the current investigation these plants have been chosen with a focus on their high polysaccharide contents. In the past years, polysaccharides were identified as compounds with multiple effects; amongst others were reduction of skin ageing (Peterszegi et al., 2003) and tissue regeneration (Biagini et al., 1991). Pivotal for the regeneration and remodeling of skin are the dermal fibroblasts of the connective tissue and the epidermal keratinocytes. Fibroblasts maintain the dermal matrix, while keratinocytes form the cornified layer by a balanced process of proliferation and differentiation (Werner et al., 2007). *In vitro* studies allow to investigate the particular influence of plant extracts and water-soluble compounds on the proliferation and metabolic activity of normal human skin fibroblasts and keratinocytes.

In previous studies, the effectiveness of extracts or single compounds was tested only for short incubations (24 h and 48 h) *in vitro*. Whereas in practice creams and ointments are applied every day (PubMed database, accessed 1st February 2012, terms: incubation time, *in vitro* test). Therefore, the purpose of this study was to elucidate the effect of a common ice plant extract and cactus pear polysaccharides in a time and concentration dependent manner on human skin fibroblasts and keratinocytes *in vitro*.

2. Materials and methods

2.1. Isolation and characterization of water soluble cactus pear polysaccharides

110 g of powdered cactus pear leaf (*Opuntia ficus-indica*, [L.] Mill., D'Arrigo Bros., Salinas, Ca, USA) was exhaustively extracted 3 times each with 640 mL Aqua Millipore® under permanent stirring at 4 °C. A reference specimen (OP1104/D) is stored at the Institute of Pharmaceutical Biology and Phytochemistry in Münster. The combined water-extracts were concentrated with a rotary evaporator at 35 °C to the final volume of 305 mL and precipitated with ethanol 96% (V/V) to a final concentration of 80%. The polysaccharides from the entire precipitate (RPS) were isolated by centrifugation at 10,000 rpm, dialysed against Aqua Millipore® using cellulose membranes (MWCO 3.5 kDa) for 3 day at 4 °C.

The resulting preparations were hydrolyzed with trifluoroacetic acid (2 mol/L) at 121 °C for 60 min and analyzed by TLC on silica gel F_{254} glass plates using acetonitrile-water 80:20 (V/V) as a mobile phase. After threefold development, the monosaccharides were detected using thymol sulphuric acid spray reagent and heating at 120 °C for 5 min. Reference standards for detection of neutral carbohydrates (Monsigny et al., 1988) and uronic acids (Blumenkrantz and Asboe-Hansen, 1973) were prepared according to the monosaccharide and uronic acid composition determined via TLC. The protein contents were determined against reference concentrations of bovine serum albumin (PAA, Laboratories, Coelbe, Germany) with Coomassie brilliant blue G250 (Bradford, 1976). Each of these tests was modified for use in 96-well-microtiter plates. Uronic acid and neutral monosaccharides were identified and quantified by ion-exchange HPAEC with pulsed-amperometric detection (Bio-LC, Dionex, Idstein, Germany) with a AS50 auto sampler, GS50 gradient pump, AS50 oven and ED50 electrochemical detector on a CarboPacTM PA1 analytical column (2 mm × 250 mm), CarboPacTM PA1, guard column (2 mm × 50 mm) and Borate-TrapTM (4 mm × 50 mm) using a ternary gradient of water, 0.1 mM NaOH and 0.5 mM NaOAc.

2.2. Common ice plant extracts

For investigations on fibroblasts and keratinocytes, pressed juices from common ice plant (*Mesembryanthemum crystallinum* L.) grown in South Africa were used and the resulting extracts were passed through 1.2 μm (McPI) or 0.2 μm (McPII) filters (Table 1). The reference specimen (MP11041/D and MP11042/D) are stored at the Institute of Pharmaceutical Biology and Phytochemistry in Münster.

2.3. Cell culture

In general media and media supplements were purchased from PAA Laboratories, Coelbe, Germany. The MCDB153 medium was obtained from Biochrom, Berlin, Germany. The cells were cultivated at 37 °C in an humidified atmosphere at 5% (MEM, MCDB 153 complete) and 8% CO_2 (DMEM) according to the suppliers instruction.

Normal human dermal fibroblasts (NHDF) were isolated from human skin grafts (Pediatric Surgical Clinic, University of Muenster, Germany) of various Caucasian subjects. The studies were approved by the local ethical committee of the University of Muenster (acceptance no. 2006-117-f-S). HaCaT-keratinocytes were kindly provided by Prof. Fusenig (DKFZ, Heidelberg, Germany). Subculture of NHDF was performed in MEM high glucose, 10% FCS, 1% L-glutamine. HaCaTs were permanently cultivated in DMEM high glucose supplemented with 10% FCS, 1% penicillin/streptomycin, 1% glutamine and 1% non-essential amino acids. Both were allowed to grow to a confluence of 80% before splitting. The studies were performed on the 2nd to 6th (NHDF) and 45th–56th passage (HaCaT). Prior to treatment with the pressed juices and polysaccharides the NHDF and HaCaT were directly adapted to serum starved media; Fibroblasts to MEM high glucose, 10% SerEx® (a defined serum alternate) and 1% L-glutamine and HaCaT-keratinocytes to MCDB 153 complete medium.

2.4. Investigation of skin keratinocyte and fibroblast proliferation, cell viability and necrosis

Due to limited water solubility the cactus pear polysaccharide extracts were dissolved in Aqua Millipore® to a stock concentration of 1 mg/mL. Both cactus pear and ice plant preparations were

Table 1
Characteristics and denomination of used polysaccharide preparations.

Extract		Denomination
Pressed juice without ethanol	1.2 μm filter	**McPI**
Pressed juice without ethanol	0.2 μm filter	**McPII**
Nopal polysaccharide	Water soluble	**NwPS**
Nopal polysaccharide	Non-swelling pectin[a]	**NPec**

[a] According to Goycoolea and Cárdenas (2003).

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