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DNA-protective effects of sumach (*Rhus coriaria* L.), a common spice: Results of human and animal studies

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ABSTRACT

Sumach (*Rhus coriaria* L.) is widely used as a spice. The aim of this study was the investigation of its DNA-protective effects in humans and animals. Prevention of the formation of strand breaks and oxidized DNA bases as well as the protection against H_2O_2- and (±)-*anti*-benzo[a]pyrene-7,8-dihydro-diol-9,10-epoxide (BPDE)-induced DNA-damage were monitored in human lymphocytes in a placebo controlled trial ($N = 8$/group) with ethanolic extract of sumach (3.0 g/day, 3 days) in single cell gel electrophoresis assays. Furthermore, DNA-protective effects of sumach were monitored in different inner organs of rats under identical conditions. No alteration of DNA-migration was detectable in human lymphocytes under standard conditions, but a decrease of the tail-lengths due to formation of oxidized purines and pyrimidines (52% and 36%) was found with lesion-specific enzymes. Also damage caused by H_2O_2 and BPDE was significantly reduced by 30% and 69%, respectively. The later effect may be due to induction of glutathione *S*-transferase (GST). After the intervention, the overall GST (CDNB) activity in plasma was increased by 40%, GST-α by 52% and GST-π by 26% (ELISA). The antioxidant effects of extract are probably due to scavenging which was observed in *in vitro* experiments, which also indicated that gallic acid is the active principle of sumach. The animal experiments showed that sumach also causes protection in inner organs. Supplementation of the drinking water (0.02 g/kg per animal) decreased the formation of oxidized DNA bases in colon, liver, lung and lymphocytes; also after γ-irradiation pronounced effects were seen.

1. Introduction

It is well documented that reactive oxygen species (ROS) play a key role in the aetiology of a number of human diseases including cancer, age related disorders, neurodegenerative diseases and infertility to name a few [1–3]. Therefore, strong efforts have been made over the last decades to identify dietary components which protect against the consequences of oxidative damage and numerous ROS-protective phytochemicals have been detected [4,5].

Also spices such as rosemary, sage, oregano, thyme, ginger, chillies and sumach have been found to possess antioxidant properties [6–12] but evidence for their effects is mainly based on results obtained in *in vitro* experiments. We stressed earlier that it is problematic to extrapolate such findings to humans *in vivo* [13,14].

In the present study, we investigated the antioxidant effects of sumach (*Rhus coriaria* L.) in humans and in rats and attempted to identify the molecular mechanisms involved and to identify its active principle. Sumach is widely used in Turkey, Iran and Arab countries as a condiment, either in pure form or in combination with other spices [10]. It is also used as a herbal remedy in traditional medicine due to its analgesic, antidiarrhetic, antiseptic, anorexic and antihypergylcaemic properties [10]. Recently, several studies were published concerning the antibacterial properties of sumach preparations which may be used for food production [10–12]. A number of *in vitro* investigations were carried out in which antioxidant effects were detected. The only *in vivo* study was published by Kanai and Okano [15]. They found protective effects against CCl_4-induced damage in livers of rats with an extract which was prepared from a related species (*R. javonica*), but the high dose they used (7.5 g/kg bw) is not relevant for humans.

To find out if sumach consumption causes DNA-protective effects in humans, we conducted a placebo controlled intervention trial in which we investigated the effect of an ethanolic

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sumach extract on the formation of single and double-strand breaks, on the endogenous formation of oxidized purines and pyrimidines and on alterations of the sensitivity of lymphocytes towards DNA-damage caused by ROS and (±)-*anti*-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE) in single cell gel electrophoresis (SCGE) assays. (±)-Anti-benzo[a]pyrene-7,8-dihydro-diol-9,10-epoxide represents the ultimate reactive metabolite of benzoapyrene (B[a]P). It forms DNA adducts and it is detoxified by glutathione *S*-transferase [16]. The anti- and the syn-forms differ in regard to the location of the OH-groups on the oxiran ring. The designation (±) indicates that this compound has two enantiomers which are both found during synthesis [17–19]. BPDE was used in this study as it is known that it is detoxified by GSTs. B(a)P was not used because lymphocytes have only low CYP1A1 activities, therefore they can not activate this compound [16].

SCGE experiments are based on the measurement of DNA-migration in an electric field [20] and are increasingly used in human intervention trials [14,21]. In order to elucidate the molecular mechanisms which account for the DNA-protective effects of sumach, we also monitored the impact of extract on the activities of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX) and on glutathione *S*-transferases (GSTs). These enzymes play a key role in the detoxification of a broad spectrum of genotoxic carcinogens [22–24]. Furthermore, direct scavenging effects were monitored in *in vitro* experiments with lymphocytes and in order to identify the active principle of the extract, additional experiments were carried out with gallic acid (GA) which is one of the main constituents of sumach [10]. On the basis of earlier results obtained in *in vitro* fractionation experiments by Kosar et al. [11], we hypothesized that GA, which possesses potent antioxidant properties [25,26] may account for the DNA-protective effects of the extract.

In order to find out if sumach extract also causes protection in inner organs, we conducted a series of animal experiments with different inner organs (brain, colon, liver, lung) and peripheral lymphocytes of non-irradiated and γ-irradiated rats. DNA-migration was investigated in SCGE assays under experimental conditions identical to those in the human trial.

2. Materials and methods

2.1. Chemicals and media

Low melting agarose (LMA) and normal melting agarose (NMA) were obtained from Gibco (Paisley, UK). Gallic acid (GA, CAS no. 149-91-7), anorganic salts, 1-chloro-2,4-dinitrobenzene (CDNB), hydrogen peroxide, Trisma base, Triton X-100, dimethyl sulfoxide (DMSO), RPMI 1640, histopaque-1077, bovine serum albumin fraction V (BSA), ethidium bromide and trypan blue were purchased from Sigma–Aldrich (Steinheim, Germany). (±)-*Anti*-B[a]P-7,8-dihydrodiol-9,10-epoxide (BPDE, CAS no. 58917-67-2) was obtained from A. Seidel (Biochemisches Institut für Umweltkarzkinogene, Großhansdorf, Germany). Formamidopyrimidine glycosylase (FPG) and endonuclease III (ENDO III) were a gift of K. Angelis (Laboratory of DNA Repair, Prague, Czech Republic). The BIO-RAD® Protein Assay came from BIO-RAD® (Munich, Germany). The HEPKIT™-Alpha and the HEPKIT™-Pi were purchased from Biotrin International Ltd. (Dublin, Ireland). The SOD-Kit came from Randox Laboratories Ltd. (Ardmore, UK). The reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), glutathione disulfide (GSSG), reduced glutathione (GSH), glutatione reductase and *tert*-butyl hydroperoxide were obtained from Fluka Chemicals (Buchs, Switzerland).

2.2. Preparation of ethanolic sumach extract

Sumach (*Rhus coriaria* L.) was purchased from Salzburger Kräuterhof (Beyerhofer GesmbH, Salzburg, Austria). The ethanolic extract was prepared as described by Nasar-Abbas and Halkman [27]. Briefly, powdered plant material (160 g of dried, ground berries) was stirred with 1600 ml 30% (v/v) aqueous ethanol at room temperature (drug solvent ratio 1:10) for 24 h. The extract was filtered through a filter paper (Whatman 1, pore size 11 μm, Schleicher & Schuell, Dassel, Germany). Subsequently, the solvent was removed in a rotary vacuum evaporator (Rotavapor R-114, Büchi, Switzerland). The dried material (35.5 g) was stored at −20 °C.

2.3. Determination of the gallic acid concentration in sumach extract

Gallic acid (GA) was quantified in the extract with an HPLC-system (Hitachi AS2000, UV–Vis detector L-4250, Tokyo, Japan) according to the method of Zhu et al. [28].

2.4. Isolation of primary human lymphocytes

Ten millilitres of blood were aspired by venipuncture and centrifuged in heparinized Vacutainer tubes (Becton-Dickinson, Plymouth, UK) at 760 × *g* for 10 min (4 °C). The plasma samples were stored at −80 °C. For SCGE assays, lymphocytes were isolated with Histopaque-1077 according to the instructions of the manufacturer.

2.5. In vitro single cell gel electrophoresis experiments with human lymphocytes

The experiments were carried out according to the guidelines for SCGE experiments [20]. Freshly isolated lymphocytes (1.5×10^5 per tube) were cultured in RPMI 1640 and incubated for 60 min (37 °C) in Eppendorf tubes (Eppendorf AG, Hamburg, Germany) with different amounts of extract or with different concentrations of GA. Subsequently, the cells were washed twice with phosphate-buffered saline (PBS, 8.0 g/l NaCl, 1.15 g/l Na_2HPO_4, 0.2 g/l KCl, 0.2 g/l KH_2PO_4, pH 7.4) and treated with H_2O_2 (75 μM) for 5 min on ice [29]. The viability of the cells was determined with the trypan blue (0.4% staining solution) exclusion technique [30]. Only cultures in which the viability was ≥80% were analysed for comet formation. After lysis (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Trizma base, 1% fresh Triton X-100, 10% fresh DMSO; pH 10.0) and electrophoresis (20 min, 300 mA, 25 V, at 4 °C, pH ≥ 12.6), the gels were stained with ethidium bromide (20 μg/ml). DNA-migration (tail-lengths and tail-moments) was monitored by use of an automated image analysis system [31].

2.6. Design of the human study

The study was approved by the Ethics Committee of the Medical University of Vienna and informed consent was obtained from all participants. Sixteen healthy volunteers (eight males; mean age: 32 ± 5 years, bw: 68 ± 3 kg, BMI: 21.8 ± 3.0, eight females; mean age: 32 ± 5 years, bw: 60 ± 4 kg, BMI: 20.3 ± 4.1) who were all non-smokers, non-vegetarians and nonusers of medications and dietary supplements participated and were randomly allocated either to the placebo group or to the sumach group. All of them filled out a questionnaire before the start of the study. Seven days before and during the intervention, all volunteers consumed a controlled diet avoiding polyphenol-rich foods and spices [32]. Additionally, they were also asked not to perform excessive physical exercise which may cause comet formation [33]. During the intervention, each participant consumed 3.0 g sumach extract daily dissolved in 500 ml drinking water over 3 consecutive days. The participants of the placebo group received the same amount of water (supplemented with 1.0 ml of grape juice/500 ml to obtain the same colour as the sumach supplemented water). Blood samples (2 × 10 ml) were collected in heparinized tubes (BD Vacutainer, Plymouth, UK) immediately before (T0) and after (T1) the intervention.

2.7. Single cell gel electrophoresis assays with human lymphocytes before and after intervention with sumach

The cells were analysed after electrophoresis under standard conditions (20 min, 300 mA, 25 V, at 4 °C, pH ≥ 12.6) which enables the detection of single- and double-strand breaks and apurinic sites as described by Tice et al. [20].

Additionally, nuclei were treated either before lysis with H_2O_2 (75 μM, 5 min, on ice) or after lysis with the lesion-specific enzymes formamidopyrimidine glycosylase (FPG) and endonuclease III (ENDO III). To determine the optimal amounts of the enzymes for the measurement of oxidized bases, calibration experiments were carried out before the intervention study with blood cells from a single donor according to the protocol of Collins et al. [34] (data not shown).

After lysis of the cells, the slides were washed twice in enzyme buffer (0.1 M KCl, 40 mM Hepes, 0.5 mM EDTA, 0.2 g/l BSA, pH 8.0) for 8 min. Subsequently, 50 μl of FPG or ENDO III (1.0 μg/ml) was added to the nuclei. The incubation time for FPG was 30 min and for ENDO III 45 min at 37 °C, respectively. After the treatment, comet formation was measured under standard conditions. In all these experiments, we also included measurements in which the nuclei were treated with the specific enzyme buffer in parallel.

Experiments with (±)-*anti*-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE) were carried out as described by Steinkellner et al. [35]. Briefly, human lymphocytes were exposed to 0.4 μM BPDE dissolved in dimethyl sulfoxide (DMSO) for 30 min at 37 °C. Subsequently, the cells were washed with PBS (pH 7.4), centrifuged (8 min, 110 × *g*) and transferred to agarose-coated slides (1% normal melting and 0.5% low agarose) and lysed. Subsequently, electrophoresis was carried out under standard conditions.

The viability of the cells was determined in each experiment with the trypan blue dye exclusion technique [30]. Comet formation was only analysed in cells from cultures in which the viability was ≥80%, as acute toxic effects may cause false positive results [36].