JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 7465–7471 7465
DOI:10.1021/jf100082p

Polyphenolic Compounds from *Salvia* Species Protect Cellular DNA from Oxidation and Stimulate DNA Repair in Cultured Human Cells

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DNA damage can lead to carcinogenesis if replication proceeds without proper repair. This study evaluated the effects of the water extracts of three *Salvia* sp., *Salvia officinalis* (SO), *Salvia fruticosa* (SF), and *Salvia lavandulifolia* (SL), and of the major phenolic constituents, rosmarinic acid (RA) and luteolin-7-glucoside (L-7-G), on DNA protection in Caco-2 and HeLa cells exposed to oxidative agents and on DNA repair in Caco-2 cells. The comet assay was used to measure DNA damage and repair capacity. The final concentration of each sage extract was 50 μg/mL, and concentrations of RA and L-7-G were 50 and 20 μM, respectively. After a short incubation (2 h), L-7-G protected DNA in Caco-2 cells from damage induced by H_2O_2 (75 μM); also, after a long incubation (24 h), SF, RA, and L-7-G had protective effects in Caco-2 cells. In HeLa cells, SO, SF, and RA protected against damage induced by H_2O_2 after 24 h of incubation. Assays of DNA repair show that SO, SF, and L-7-G increased the rate of DNA repair (rejoining of strand breaks) in Caco-2 cells treated with H_2O_2. The incision activity of a Caco-2 cell extract on a DNA substrate containing specific damage (8-oxoGua) was also measured to evaluate effects on base excision repair (BER) activity. Preincubation for 24 h with SO and L-7-G had a BER inductive effect, increasing incision activity in Caco-2 cells. In conclusion, SO, SF, and the isolated compounds (RA and L-7-G) demonstrated chemopreventive activity by protecting cells against oxidative DNA damage and stimulating DNA repair (SO, SF, and L-7-G).

KEYWORDS: Chemoprevention; DNA damage; DNA repair; sage water extracts; polyphenolic compounds

INTRODUCTION

Carcinogenesis is a multistep process involving mutations in critical genes required for maintaining cellular homeostasis. DNA damage can lead to mutations and carcinogenesis if replication proceeds without proper repair. Chemoprevention is an approach to controlling cancer that consists in the use of natural products or synthetic chemical agents to prevent, reverse, or suppress malignancy before the development of invasive cancer (*1*). Accumulating scientific evidence shows that altering dietary habits is an effective approach for reducing cancer risk and for modifying the biological behavior of tumors. Epidemiological and experimental studies show that plant foods, namely, fruits, vegetables, beverages, medicinal plants and herbs, and some of their phytochemical constituents such as phenolics, flavonoids, and carotenoids confer protection against a wide range of cancer types including colon cancer (*2*−*7*). Several mechanisms have been suggested to explain the chemopreventive effects of phytochemicals such as inhibition of carcinogen uptake into the cells, induction of detoxification enzymes, prevention of DNA damage, enhancement of DNA repair, increased anti-inflammatory activity, inhibition of cell proliferation, and modulation of apoptosis and signal transduction pathways (*1*, *2*, *8*, *9*).

Oxidative stress appears as a result of an imbalance between the production of free radicals and antioxidant defenses. Free radicals such as reactive oxygen species (ROS) besides damaging cellular molecules such as proteins and lipids can also cause the formation of strand breaks and pyrimidine- and purine-derived lesions in DNA, leading to genome destabilization (*10*). Guanine is the base most susceptible to oxidative attack, and 8-oxoGua is one of the most abundant lesions; it can be mutagenic if not repaired before DNA replication (*11*). Cells have a number of pathways that recognize and repair different types of lesions and maintain genomic integrity. Oxidatively damaged bases are preferentially repaired by the base excision repair (BER) pathway (*12*).

Sage (*Salvia* sp.) plants are well-known for their use in traditional medicine. As a result of the health-improving effects of *Salvia officinalis* (common sage), the use of this plant is referred to from Ancient Roman times through the Middle Ages. Sentences such as "Why should a man die while sage grows in his garden?" reflect the importance of this plant in traditional

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Published on Web 05/20/2010

medicine. However, experimental studies that support its medicinal properties are scarce. Sage plants are rich in phenolic compounds, some of them with biological activities including antioxidant, cytoprotective, antiproliferative, and genoprotective properties that could be responsible for the pharmacological effects of these plants (*13*−*16*). Although the antioxidant properties of *S. officinalis* have been moderately studied, effects of other sage plants such as *Salvia fruticosa* and *Salvia lavandulifolia*, also frequently used, are poorly studied. Our knowledge about the effects of these plant extracts on DNA protection and DNA repair is still limited.

In this study, we evaluated the effects of three *Salvia* sp. water extracts, *Salvia officinalis* (SO), *Salvia fruticosa* (SF), and *Salvia lavandulifolia* (SL), and the phenolic constituents rosmarinic acid (RA) and luteolin-7-glucoside (L-7-G) on DNA protection in two human cell lines, Caco-2 and HeLa, exposed to oxidative agents. We also evaluated effects of the plant extracts and phenolic compounds on DNA repair in Caco-2 cells. Caco-2 cells were chosen to illustrate the possible involvement of cellular biotransformation in the effects of the test extracts and compounds, which may explain their chemopreventive effects on colon cancer. In contrast to HeLa cells, Caco-2 cells retain some of the xenobiotic metabolizing pathways of colonic epithelium (*17*). Comparison between results with the two cell lines will reveal the role of bioactivation for the effects of the compounds.

MATERIAL AND METHODS

Chemicals. Rosmarinic acid, hydrogen peroxide, Dulbecco's Modified Eagle Medium (DMEM), antibiotic solution, and trypsin solution were purchased from Sigma-Aldrich (St. Louis, MO). Luteolin-7-glucoside was from Extrasynthese (Genay, France). Fetal bovine serum (FBS) was purchased from Biochrom KG (Berlin, Germany). Ro (photosensitizer Ro19-8022) was a gift from F. Hoffmann-La Roche (Basel, Switzerland). SYBR Gold (nucleic acid gel stain) was from Invitrogen Molecular Probes (Eugene, OR). All other reagents and chemicals used were of analytical grade. Stock solutions of sage water extracts and RA were made in water, whereas Lut-7-G was prepared in dimethyl sulfoxide (DMSO) and aliquots kept at −20 °C.

Plant Material and Preparation of Sage Extracts. *S. officinalis* L., *S. fructicosa* L., and *S. lavandulifolia* plants were cultivated in an experimental farm located in Arouca, Portugal, and were collected between 2001 and 2003. Water extracts were prepared with aerial parts of plants as described previously by Lima et al. (*13*). In brief, 150 mL of ultrapure Milli-Q boiling water was poured over 2 g of dried aerial plant material and allowed to steep for 5 min. The filtered water extracts were lyophilized to dryness and kept at −20 °C until further use. The yield of water extracts was 25.8% (w/w) for SO, 19.1% (w/w) for SF, and 17% (w/w) for SL, in terms of initial crude plant material dry weight.

Cell Culture. Caco-2 cells (derived from human colon carcinoma) and HeLa cells (derived from human cervical cancer) were maintained as monolayer cultures in DMEM supplemented with 10% FBS and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin), under an atmosphere of 5% CO_2 at 37 °C. Cells were trypsinized when nearly confluent.

Cells were seeded onto 12-well plates, with 1 mL/well at a density of 0.2×10^6 cells/mL. Incubations with different concentrations of test compounds and extracts were made in complete DMEM medium. L-7-G was first dissolved in DMSO (final concentration below 0.5%). Hydrogen peroxide and RA as well as sage lyophilized water extracts were dissolved in PBS, and controls received DMSO or PBS, respectively.

Genotoxic Effects of Sage Water Extracts and Test Compounds. Caco-2 and HeLa cells were incubated for 24 h at 37 °C with sage water extracts or test compounds at different concentrations. Cells were collected by trypsinization, and DNA damage (strand breaks, SBs) was evaluated by the alkaline version of the comet assay. Inclusion of digestion with formamidopyrimidine DNA glycosylase (FPG) allowed assessment of effects on oxidized purines.

Effects of Sage Water Extracts and Test Compounds on Oxidative DNA Damage. To evaluate protection against oxidative damage, Caco-2 and HeLa cells were preincubated for 24 h (long incubation) or 2 h (short incubation) at 37 °C, with lyophilized water extract of each plant dissolved in PBS, or with test compounds, added to culture medium. The final concentration of each sage extract was 50 μg/mL, and concentrations of RA and L-7-G were 50 and 20 μM, respectively. The concentrations of test compounds used in this study are of the same magnitude as are present in sage water extracts (*13*). Cells were washed with PBS and treated with H_2O_2 (75 μM) (5 min on ice) to induce SBs or with 1 μM Ro plus visible light from a 500 W tungsten−halogen source (1.5 min on ice) at 33 cm to induce 8-oxoGua. DNA damage (SBs and 8-oxoGua) was evaluated by the comet assay without or with FPG, respectively.

Comet Assay. The alkaline version of the single cell gel electrophoresis assay was used to evaluate DNA damage as previously described by Lorenzo et al. (*18*), with some modifications. Briefly, Caco-2 cells were trypsinized, washed, and centrifuged, and the pellet was suspended in low melting point agarose; about 2×10^4cells was placed on a slide precoated with normal melting point agarose and covered with a coverslip. After 10 min at 4 °C, the coverslips were removed and slides were placed in lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, pH 10) plus 1% Triton X-100 for 1 h at 4 °C. When oxidized bases were to be measured, slides after lysis were washed three times with enzyme buffer (40 mM HEPES, 0.1 M KCl, 0.5 mM EDTA, 0.2 mg/mL BSA, pH 8.0) and incubated with 30 μL of FPG in this buffer or with buffer only for 20 min at 37 °C. Slides were then placed in a horizontal electrophoresis chamber with electrophoresis solution (300 mM NaOH, 1 mM Na_2EDTA, pH 13) for 30 min at 4 °C for the DNA to unwind before electrophoresis was run for 30 min at 25 V and ∼300 mA. After electrophoresis, slides were washed two times with PBS and dried at room temperature. For analysis of the comet images, slides were stained with SYBR Gold solution for 30 min at 4 °C; after drying, slides were analyzed in a fluorescence microscope and the Comet 4 image analysis system (Perceptive Instruments) was used to calculate the parameter percent tail intensity.

Effects of Sage Water Extracts and Test Compounds on Repair Ability. To assess effects of sage water extracts or test compounds on DNA repair ability, we used two different methods: the "cellular repair assay" that measures the ability of cells to rejoin strand breaks induced by H_2O_2 and the "in vitro BER assay" that measures the ability of cells to repair oxidized bases. In repair assays only Caco-2 cells were used.

Cellular Repair Assay. In the cellular repair assay two different treatment regimens were used: (1) Pretreatment with sage water extracts (50 μg/mL) or test compounds (50 μM RA; 20 μM L-7-G) was followed by exposure to H_2O_2 and recovery in fresh medium. Briefly, Caco-2 cells were preincubated with sage water extracts or test compounds for 24 h at 37 °C. Cells were washed with PBS and treated with H_2O_2 (75 μM) for 5 min on ice to induce SBs. H_2O_2 was removed, and cells were washed with PBS and then incubated in fresh culture medium for 0, 10, 30, or 60 min at 37 °C. Thus, we evaluated the effect of preincubation in test extract/compound on the ability of cells to rejoin SBs. (2) H_2O_2 treatment was done before cells were incubated with the test extract/compounds. Briefly, Caco-2 cells were treated with H_2O_2 (75 μM) for 5 min on ice to induce SBs. Cells were washed with PBS to remove H_2O_2 and then incubated with sage water extracts or test compounds for 0, 10, 30, or 60 min at 37 °C. The aim was to test effects on nonenzymatic repair.

In Vitro BER Assay. This assay measures the excision repair activity of an extract prepared from cells treated with test compounds or plant extract by providing the cell extract with a DNA substrate containing specific damage. In this case the substrate DNA was from cells previously exposed to Ro plus visible light to induce 8-oxoGua that is repaired by BER, and it was prepared as described by Gaivão et al. (*19*). Incision at damage sites is detected using the alkaline comet assay and indicates repair ability.

Cell extracts and DNA substrate were preparated as described previously by Lorenzo et al. (*18*), with some modifications. Briefly, for cell extract preparation, Caco-2 cells were incubated with sage water extracts (50 μg/mL) or test compounds (50 μM RA; 20 μM L-7-G) for 24 h at 37 °C. Cells were washed with PBS, trypsinized, and resuspended in PBS. Cells were divided in aliquots with 1×10^6cells/mL, and after centrifugation, the dry pellets were frozen in liquid nitrogen and stored at −80 °C. For substrate preparation, HeLa cells near confluence were treated with Ro plus visible light (5 min at 33 cm on ice) to induce 8-oxoGua. Cells were washed with PBS, trypsinized, and resuspended in medium. Cells were

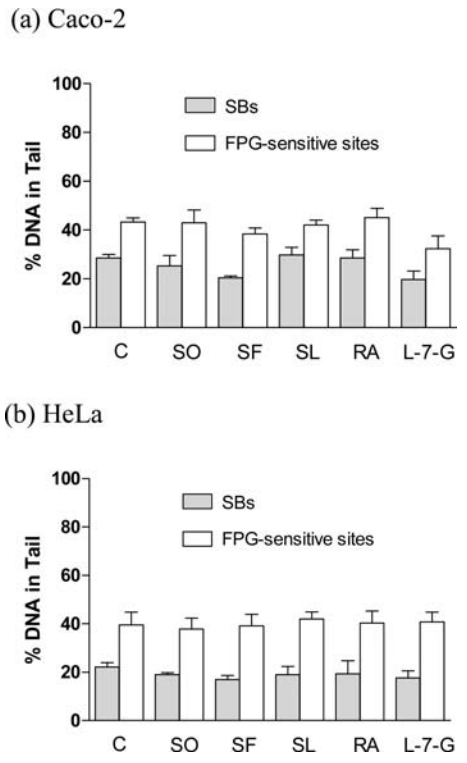

Figure 1. DNA damage (SBs and FPG-sensitive sites) in Caco-2 and HeLa cells treated for 24 h with sage water extracts (50 μg/mL), RA (50 μM), and L-7-G (20 μM). Results are expressed as mean ± SEM of three independent experiments.

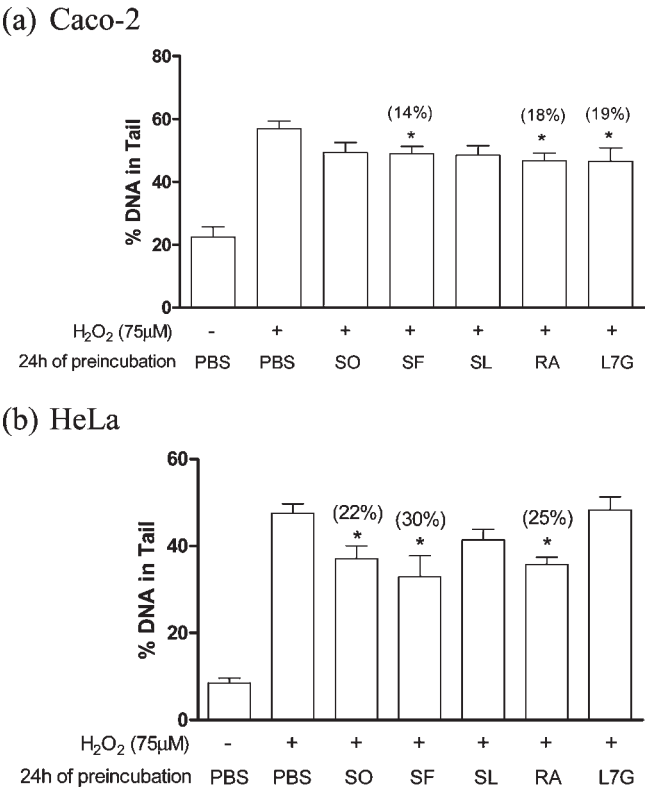

Figure 2. Effects of 24 h of treatment with SO, SF, SL (50 μg/mL), RA (50 μM), or L-7-G (20 μM) on DNA damage induced by 75 μM H_2O_2 (5 min, on ice) in Caco-2 (**a**) and HeLa cells (**b**). Results are expressed as mean ± SEM of at least four independent experiments.

centrifuged, the pellet was resuspended in freezing medium (DMEM supplemented with 20% FBS and 10% DMSO), and aliquots of 1×10^6cells/mL were frozen slowly and kept at −80 °C. HeLa cells without Ro treatment were also frozen in freezing medium and kept at −80 °C.

On the day of the experiment, extracts were resuspended in 65 μL of extraction buffer (45 mM Hepes, 0.4 M KCl, 1 mM EDTA, 0.1 mM dithiothreitol, and 10% glycerol, pH 7.8) plus Triton X-100 (0.25%), mixed for 5 s on a vortex at top speed, and incubated for 5 min on ice. After centrifugation (~14000*g*, 4 °C, 5 min), 55 μL of supernatant was removed and mixed with 220 μL of cold reaction buffer. Two gels per slide containing 2×10^4 substrate cells/gel (with or without treatment with Ro plus visible light) were placed on slides precoated with normal melting point agarose and lysed for at least 1 h. Slides were washed three times with reaction buffer, and 30 μL of extract was added to each gel and incubated for 20 min at 37 °C in a moist box. FPG and reaction buffer were added to separate gels as positive and negative controls, respectively. After the 20 min incubation, slides were transferred immediately to alkaline electrophoresis solution, and the normal comet assay was run.

Statistical Analysis. Results were expressed as mean ± SEM. Significant differences ($P < 0.05$) were evaluated by Student's *t* test.

RESULTS

Genotoxicity of Sage Water Extracts and Test Compounds. We first examined the effects of sage water extracts (SO, SF, and SL) and test compounds (RA and L-7-G) on induction of SBs and oxidized bases. For this, Caco-2 and HeLa cells were incubated with sage water extracts or test compounds for 24 h at 37 °C, and DNA damage was assessed by the comet assay with and without FPG treatment. At tested concentrations sage water extracts and test compounds did not induce either SBs or oxidized purines (FPG-sensitive sites) in either cell line (Figure**1**).

Effects of Sage Water Extracts and Test Compounds on Oxidatively Induced DNA Damage. To evaluate possible effects of sage water extracts and test compounds on oxidatively induced DNA damage, we incubated Caco-2 and HeLa cells for a long (24 h) or short (2 h) period with extracts or compounds before treatment with H_2O_2 or Ro plus visible light.

In the case of H_2O_2-treated Caco-2 cells, SF, RA, and L-7-G had a significant protective effect after a long period of incubation (24 h) at the tested concentrations (**Figure 2a**). In HeLa cells SO, SF, and RA show a strong protective effect against H_2O_2-induced DNA damage after a long period of incubation (**Figure 2b**). With a short period of incubation (2 h), L-7-G markedly decreased DNA SBs induced by H_2O_2 in Caco-2 cells, and SF shows a tendency to protect DNA (around 26% of protection ($P = 0.09$)) (**Figure 3a**). However, in HeLa cells only SL shows a tendency to protect DNA (around 21% of protection) (**Figure 3b**). In the assay with Ro plus light, Caco-2 and HeLa cells were also pretreated for a long or short period with sage water extracts or test compounds. With a long period of incubation, sage water extracts and test compounds did not protect DNA from damage induced by Ro plus visible light in either cell line (**Figure 4**). However, SF ($P = 0.09$) and L-7-G ($P = 0.08$) show a tendency to protect DNA in Caco-2 cells (**Figure 4a**). With a short incubation only RA significantly decreased oxidized DNA bases induced by Ro plus visible light in Caco-2 cells, by 39% (**Figure 5a**).

Effects of Sage Water Extracts and Test Compounds on Repair Ability. *Cellular Repair Assay*. The ability of Caco-2 cells to rejoin strand breaks induced by H_2O_2 was measured at different times (0, 10, 30, and 60 min). SBs decreased with the time of recovery, and at 60 min the levels of SBs were similar to the control (without H_2O_2 treatment) (data not shown). To assess effects of sage water extracts and test compounds on the ability of Caco-2 cells to rejoin strand breaks, two different treatments were used. (1) Cells were treated with sage water extracts or with test compounds for 24 h before H_2O_2 exposure and allowed to recover

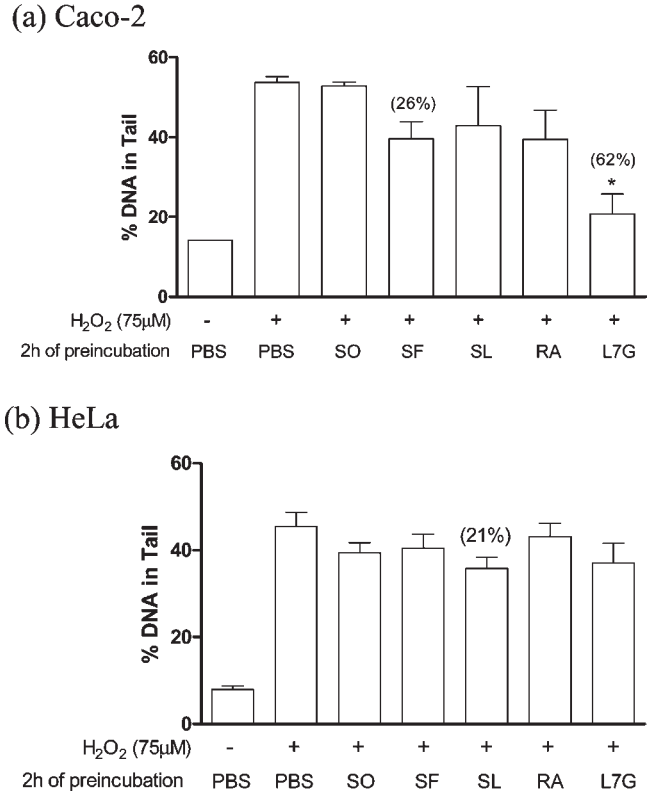

Figure 3. Effects of 2 h of treatment with SO, SF, SL (50 μg/mL), RA (50 μM), or L-7-G (20 μM) on DNA damage induced by 75 μM H_2O_2 (5 min, on ice) in Caco-2 (**a**) and HeLa cells (**b**). Results are expressed as mean ± SEM of at least three independent experiments.

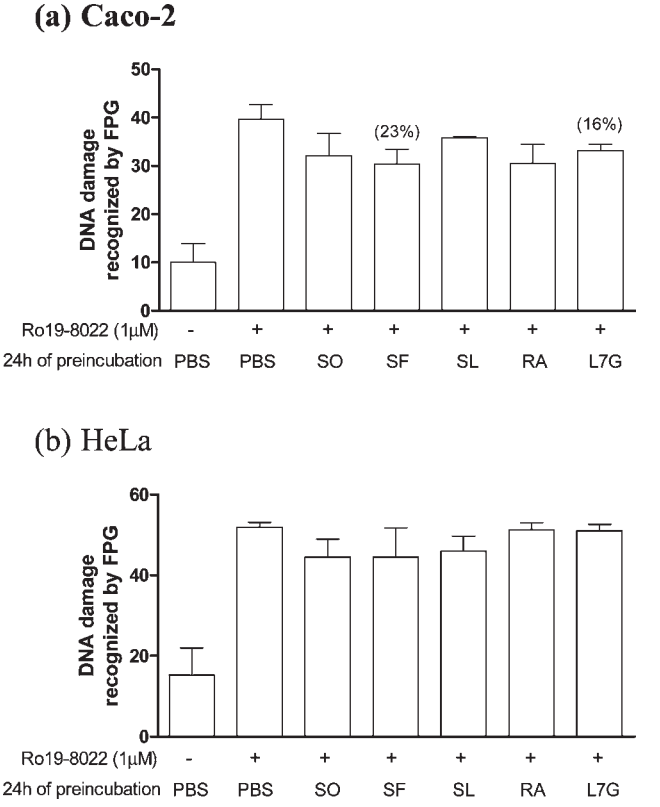

Figure 4. Effects of 24 h of treatment with SO, SF, SL (50 μg/mL), RA (50 μM), or L-7-G (20 μM) on DNA damage induced by 1 μM Ro19-8022 (1.5 min, on ice) plus light in Caco-2 (**a**) and 1 μM Ro19-8022 (2.5 min, on ice) plus light in HeLa cells (**b**). Results are expressed as mean ± SEM of at least three independent experiments.

in fresh medium for 30 min at 37 °C. For recovery time we chose 30 min because it is within the approximately linear phase of SB repair. Caco-2 cells treated only with H_2O_2 (control cells), after 30 min of recovery, had rejoined ~50% of SBs. Cells preincubated with SO or SF had rejoined 84 and 92%, respectively, representing relative increases in the extension of DNA rejoining of 62 and 86% compared with the control cells, respectively. L-7-G (P = 0.08) also seems to increase the ability to rejoin SBs (**Figure 6**). (2) Cells were incubated with sage water extracts and test compounds for different times after H_2O_2 exposure. No difference was seen in SB rejoining in comparison with cells incubated with fresh medium after H_2O_2 exposure (data not shown).

In Vitro BER Assay. The ability of Caco-2 cells to repair oxidized bases by BER was measured by a modified comet assay, "in vitro base excision repair assay". In this assay a DNA substrate containing specific damage, 8-oxoGua, induced by Ro plus visible light, was incubated with an extract of Caco-2 cells. To prepare the Caco-2 cell extract, cells were pretreated with sage water extracts or test compounds for 24 h at 37 °C. An increase of SBs in substrate DNA detected by the comet assay indicates an increase in excision repair activity of extract. **Figure 7** shows breaks in the substrate DNA when treated with FPG (positive control) increased compared with breaks in the substrate incubated only with reaction buffer (negative control). Extracts from Caco-2 cell treated only with PBS or DMSO gave increased SBs when compared with the negative control. This indicates that Caco-2 cells have detectable excision repair activity. Extracts obtained from cells pretreated with 50 μg/mL SO or 10 μM L-7-G had significantly increased excision repair activity when compared with an extract of Caco-2 cells treated with PBS or DMSO, by 27 and 20%, respectively. SL (P = 0.06) and RA (P = 0.06) also tend to increase excision repair activity. These results indicate that these compounds/extracts enhance DNA repair activity in Caco-2 cells. These extracts did not increase SBs when incubated with substrate without 8-oxoGua lesions (data not shown), indicating that the increase of breaks observed for SO, L-7-G, SL, and RA corresponds to recognition of 8-oxoGua lesions and demonstrating the absence of nonspecific nucleases in cell extracts.

DISCUSSION

Living cells are constantly exposed to potentially damaging chemical and physical agents, the origin of which may be intracellular or extracellular. Of particular interest are the ROS that induce oxidative damage in DNA, lipids, and proteins. Antioxidant defense systems have evolved, which protect cells from oxidative damage; they comprise enzymatic components such as catalases and nonenzymatic components such as glutathione. However, these systems are not enough to avoid some DNA damage, and cells have DNA repair pathways that recognize and repair different types of lesions. Natural compounds present in the diet can improve cellular defenses by acting directly on ROS or by stimulating endogenous defense systems (*10*, *11*, *20*, *21*).

In this study, we evaluated the effects of three *Salvia* sp. water extracts, SO, SF, and SL, and phenolic constituents, RA and L-7-G, on DNA protection in Caco-2 and HeLa cells and induction of DNA repair in Caco-2 cells exposed to oxidative agents. On DNA protection, after a short period with extracts or compounds (2 h), L-7-G showed a strong protective effect against H_2O_2-induced DNA damage in Caco-2 cells. SF and SL also protected DNA against this damage (26 and 21% protection in Caco-2 and HeLa, respectively), although with marginal statistical

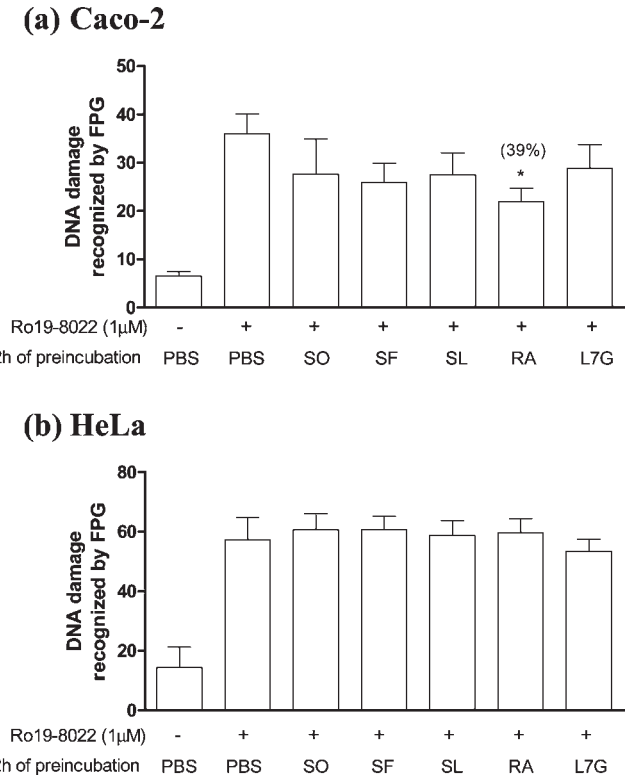

Figure 5. Effects of 2 h of treatment with SO, SF, SL (50 μg/mL), RA (50 μM), or L-7-G (20 μM) on DNA damage induced by 1 μM Ro19-8022 (1.5 min, on ice) plus light in Caco-2 (**a**) and 1 μM Ro19-8022 (2.5 min, on ice) plus light in HeLa cells (**b**). Results are expressed as mean ± SEM of at least four independent experiments.

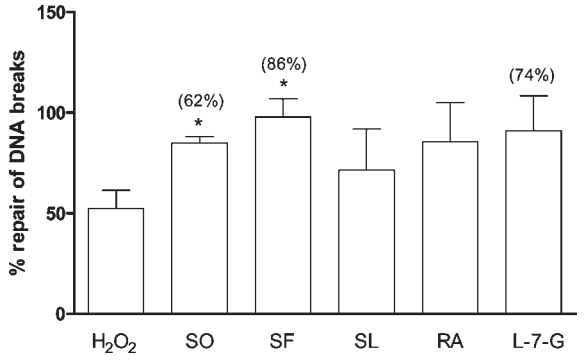

Figure 6. Cellular repair of H_2O_2-induced damage in Caco-2. Results are expressed as mean ± SEM of at least three independent experiments.

significance. At a long period of incubation (24 h), SF, RA, and L-7-G showed a protective effect against H_2O_2-induced DNA damage; however, for SF and L-7-G, protection is less pronounced than after a short period in Caco-2 cells. In contrast, in HeLa cells, SO, SF, and RA show a greater protection after a long period of incubation when compared with a short incubation. This difference can reflect differences in uptake of compounds and metabolic activity of both cell lines; Caco-2 cells are known to retain phase I and phase II metabolizing activities (*17*) and therefore may be transforming the compounds/extracts in nonactive compounds. Chemopreventive effects on colon cancer of these sage extract/compounds may require frequent ingestions because their effects on colonocyte DNA protection is only transient.

A major form of oxidative damage in genomic DNA is the premutagenic lesion 7,8-dihydro-8-oxoguanine (8-oxoGua), which

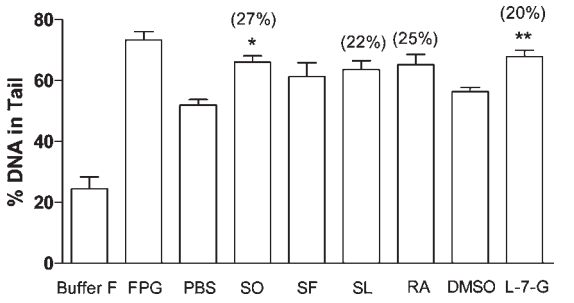

Figure 7. In vitro DNA repair: incision by Caco-2 cell extracts incubated for 20 min with gel-embedded nucleoid DNA containing 8-oxoGua lesions. Results are expressed as mean ± SEM of at least four independent experiments. PBS and DMSO refer to extracts from cells treated only with the relevant solvent vehicle (PBS for SO, SF, SL, and RA; DMSO for L-7-G).

causes G to T transversions (*22*). In this study, we exposed Caco-2 and HeLa cells to Ro plus visible light to induce 8-oxoGua. Only RA protected DNA against this damage in Caco-2 after a short period of incubation but did not protect it after a long period of incubation.

RA is the major phenolic compound in the three extracts and is most abundant in SF (577.29 μg/mL of water extract), less so in SO (362.0 μg/mL of water extract) and least in SL (146.43 μg/mL of water extract). In our study, RA showed moderate ability to protect against DNA damage induced by H_2O_2, as Lima et al. (*23*) found in HepG2 cells. It is likely that RA contributes to the protective effect observed with SF.

L-7-G is more abundant in SO (115.30 μg/mL of water extract) than in SL (29.63 μg/mL of water extract) but is not present in SF water extract. Although L-7-G shows strong protective effects in Caco-2, SO and SL did not have protective effects. Also, Lima et al. (*24*) show that water extract from *S. officinalis* did not prevent DNA damage induced by *t*-BHP. Aherne et al. (*25*) showed that *S. officinalis* L. protected against H_2O_2-induced DNA damage and cytotoxicity in Caco-2; however, differences in experimental conditions may account for the different results.

The antiradical activity of RA is higher than that of L-7-G (*24*), but the degree of hydrophobicity is higher for L-7-G than for RA, which can explain the higher protection found in Caco-2 when pretreated with L-7-G than RA. The biological effect of polyphenols depends on the extent to which they interact with cell membranes and their uptake into the cell (*26*, *27*).

In Caco-2 cells, our compounds show a protective effect for a short period of incubation, losing this effect when incubated for a long period, presumably because the compounds are metabolized in the cell in that time.

Although effects of plant extracts and isolated compounds on DNA repair are still poorly studied, some papers show that polyphenols such as curcumin and quercetin increase DNA repair activity (*14*, *28*).

As suggested by Tan et al. (*29*), effects of polyphenols could be due to three possible mechanisms: (1) an antioxidative mechanism in which polyphenols scavenge H_2O_2, reducing the amount of DNA oxidative damage; (2) enzymic repair, in which polyphenols promote the effect of spontaneous enzymic repair; or (3) nonenzymic repair, in which polyphenols rapidly repair the transient DNA damage resulting from H_2O_2 attack before enzymic repair initiation.

In the present study, when cells were incubated with sage water extracts or RA or L-7-G after H_2O_2 exposure, no effect on levels of DNA damage was seen, indicating that "nonenzymic repair" of damage was not occurring. However, pretreatment of cells with

SO, SF, or L-7-G did accelerate SB rejoining, indicating an induction or activation of repair enzymes.

Confirming this effect on repair enzyme activity, in whole cells, we found that SO and L-7-G increase the incision activity of Caco-2 cells extracts in the in vitro assay. Despite the fact that L-7-G is tested here in a higher concentration than that in which it is present in SO (the richest extract in L-7-G), the incision activity of this extract may reflect the effects of L-7-G.

SF and RA show a tendency to increase incision activity, although not significant statistically. Silva et al. (*30*) show that rosmarinic acid increased the repair of oxidized bases induced with the photosensitizer compound (Ro 19-8022) and had an effect on the expression of OGG1 repair gene, in PC12 neuronal cells. Guarrera et al. (*31*) show that a flavonoid-rich diet increases expression of some DNA repair genes such as XRCC3 (involved in double strand breaks repair) in healthy male smokers. However, poor correlation between the activity of DNA repair enzymes and gene expression or protein levels has been reported by several authors (*18*, *32*, *33*). Clearly, the measurement of repair enzyme activity, as in our in vitro repair assay, can give a more realistic estimation of DNA repair capacity than is provided by measurement of gene expression.

The study demonstrated chemopreventive effects of SO and SF, in agreement with their medicinal reputation. SL is ineffective, consistent with the low levels of phenolic compounds found in this plant. We suggest that dietary strategies including the consumption of plant foods (not only sage species) rich in L-7-G and RA may contribute to preventing colon cancer through decreasing the extent of oxidative damage to colonocyte DNA, and also by inducing cellular DNA repair activity.

ABBREVIATIONS USED

SO, *Salvia officinalis*; SF, *Salvia fruticosa*; SL, *Salvia lavandulifolia*; RA, rosmarinic acid; L-7-G, luteolin-7-glucoside; 8-oxoGua, 7,8-dihydro-8-oxoguanine; SBs, strand breaks; BER, base excision repair; FPG, formamidopyrimidine DNA glycosylase; Ro, photosensitizer Ro19-8022; H_2O_2, hydrogen peroxide.

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Received for review January 8, 2010. Revised manuscript received April 6, 2010. Accepted April 26, 2010. A.A.R. is supported by the Foundation for Science and Technology, Portugal, Grant SFRH/BD/35672/2007.