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Chemopreventive potential of phenolic compounds in oral carcinogenesis

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ABSTRACT

Objective: To evaluate the chemopreventive potential of phenolic compounds – potassium apigenin, cocoa, catechins, eriocitrin and rosmarinic acid in oral carcinogenesis induced in hamsters by means of the topical application of 7,12-dimethylbenz(a)anthracene(DMBA).
Study design: An experimental study at the University of Murcia.
Methods: 50 male Syrian hamsters (*Mesocricetus auratus*) were divided into five groups of ten: Group I (control group): 0.5% DMBA; Group II: 0.5% DMBA + 1.1 mg/15 ml potassium apigenin; Group III: 05% DMBA + 2.5 mg/15 ml cocoa catechins; Group IV: 0.5% DMBA + 6 mg/15 ml eriocitrin; Group V: 0.5% DMBA + 1.3 mg/15 ml rosmarinic acid. The flavonoids were administered orally. All the animals were sacrificed after 12 weeks. Macroscopic, microscopic and immunohistochemical (PCNA and p53) analyses of the lesions were performed.
Results: All the groups treated with phenolic compounds showed lower incidences of tumour, greater differentiation and lower scores in the tumour invasion front grading system in comparison with the control group. Potassium apigenin and rosmarinic acid achieved the best results, the former considerably reduced the carcinoma tumour volumes developed and both significantly reduced the intensity and aggression of the tumours. Immunoexpression of PCNA and p53 were significantly altered during DMBA-induced oral carcinogenesis.
Conclusions: Animals treated with phenolic compounds, particularly potassium apigenin and rosmarinic acid, showed a lower incidence of tumours.

1. Introduction

Oral squamous cell carcinoma is the most frequently occurring cancer of the head and neck and is characterized by poor prognosis and a low patient survival rate.[1,2]

Recent efforts to control the incidence of oral squamous cell carcinoma, have focused on developing effective chemopreventive strategies. Chemoprevention by natural products and dietary and lifestyle changes have evolved as promising strategies for the management of cancer. In this way, dietary phytochemicals have gained significant recognition in recent

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years as potential candidates for cancer chemoprevention.[1–9] *In vitro* studies have shown that flavonoids significantly increase p53, E-cadherin and β-catenin protein expression and caspase 3, fas ligand and Fas/Apo-1 receptor activation which are strongly associated with the signal transduction pathways involved in apoptosis and cellular adhesion that affect the chemosensitivity of tumour cells. The biological behaviour of flavonoids depends on chemical structure and epidemiological studies support their role in cancer prevention.[10–14]

Apigenin, a flavonoid compound, has the capacity to inhibit the proliferation and cell viability in numerous cancer cell lines.[14–16] Apigenin has shown chemoprotective effects in various *in vivo* mouse models of cancer (lung, skin, neck of the uterus, ovarian, prostate, leukaemia, and colon), in which it has been seen to reduce the size of primary tumors.[17] It is also able to inhibit the mobility of tumour cells (inhibition of FAK expression)[18] and to reduce the number and size of metastatic nodes in ovarian cancer, breast cancer, and melanoma.[19]

Initial observations have shown that procyanidin-rich fractions prepared from the seeds of Theobroma cacao, which are used to prepare cocoa, inhibit *in vitro* growth of several human breast cancer cell lines.[20] This research has revealed oligomeric procyanidins, such as the pentameric procyanidin, to be cytotoxic to breast cancer cells.

Rosmarinic acid (RA) ((2R)-2-[[(2E)-3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]]oxy-3-(3,4-dihydroxyphenyl) propanoic acid) has a number of interesting biological activities, including antiviral, antibacterial, anti-inflammatory and antioxidant effects.[21–23] RA prevents mutagenic activity, which has been observed by means of micronuclear tests in Swiss mice. In addition, the reduction in tumorigenesis in a murine two-stage skin carcinogenesis model with topical application of *Perilla Frutescens* extract has also been attributed to the presence of RA. It has been shown to delay colorectal carcinogenesis in rodent models. The pharmacological effect of RA has been seen to act through the inhibition of several complement-dependent inflammatory processes. Recent studies confirm that caffeoyl esters such as RA show high specific antioxidant activity, delay vitamin E depletion, decrease pro-inflammatory lysophosphatidyl choline production and prevent the oxidation of low-density lipoprotein (LDL), which is compatible with its anti-inflammatory and anti-atherosclerotic role in pathophysiological conditions.[21–23]

Among the glycoside flavonoids present in lemons, eriocitrin shows the highest antioxidant activity and has been shown to inhibit proliferation and apoptosis in different cancer cell lines.[24–26] Ogata et al.[27] demonstrated that lemon flavonoids and their metabolites induce apoptosis in leukaemia cell lines HL-60 dose- and time-dependently.

The aim of this study was to evaluate the chemopreventive efficacy of phenolic compounds potassium apigenin, cocoa catechins, eriocitrin and rosmarinic acid on oral carcinogenesis induced in hamsters by means of topical application of DMBA.

2. Materials and methods

2.1. Animals

The study used 50 male Syrian hamsters (*Mesocricetus auratus*), with an average weight of 100 g and an average age of five months. All the animals were supplied by the University of Murcia (Spain) Research Support Services Animal Laboratory (License No. REGAES 300305440012). The hamsters were housed in cages and received food and water *ad libitum*. The room in which they were housed had a controlled 12/12 light/darkness cycle and a temperature of 22 °C, in compliance with European Union norms for the protection of animals used in experimentation (2010/63/UE). The experiment was approved by the University of Murcia Bioethics Committee.

2.2. Agents

- Polycyclic aromatic hydrocarbon 7,12-dimethyl-1,2-bezantracene (DMBA) (Sigma Aldrich Co., Madrid, Spain). The DMBA was dissolved in acetone (Sigma Aldrich Co., Madrid, Spain) at 0.5%. The anaesthetic used was ketamine (Imalgene 1000 Merial, Barcelona, Spain) and xylazine (Xylagesic 2% [20 mg/ml] Laboratorios Calier S.A, Barcelona, Spain).
- Concentrated extract of apigenin (90% HPLC purity) (Nutrafur, S.A., Murcia, Spain) dissolved in distilled water at a concentration of 1.1 mg/15 ml.
- Concentrated extract of cocoa catechins (40% HPLC purity) (Nutrafur, S.A., Murcia, Spain) dissolved in distilled water at a concentration of 2.5 mg/15 ml.
- Concentrated extract of eriocitrin (20% HPLC purity) (Nutrafur, S.A., Murcia, Spain) dissolved in distilled water at a concentration of 6 mg/15 ml.
- Concentrated extract of rosmarinic acid (15% HPLC purity) (Nutrafur, S.A., Murcia, Spain) dissolved in distilled water at a concentration of 1.3 mg/15 ml.

2.3. Experiment procedure

Fifty animals were divided randomly into five groups of ten: Group I (control group) treated with 0.5% DMBA in acetone ($n = 10$); Group II treated with 0.5% DMBA + concentrated extract of potassium apigenin ($n = 10$); Group III treated with 05% DMBA + concentrated extract of cocoa catechins ($n = 10$); Group IV treated with 0.5% DMBA + concentrated extract of eriocitrin ($n = 10$); Group V treated with 0.5% DMBA + concentrated extract of rosmarinic acid ($n = 10$). All the phenolic compounds were administered *via* the animals' drinking water, consumed *ad libitum*. The animals in the study groups treated with polyphenols received pre-treatment for two weeks prior to administering the carcinogenic agent so that the animals would have plasma and tissular concentrations of each of the compounds.

When the pretreatment period came to an end, the carcinogenic agent DMBA dissolved in acetone was administered topically, for which the animals were sedated three times a week by means of a mixture of ketamine and xylazine (50%). This was applied to the left cheek mucosa, as follows: the area was dried with a cotton swab, and 40 μl (200 μg) of DMBA solution was applied over a 1-min period using a micropipette. After 12 weeks of DMBA application, the animals were euthanized using a CO_2 chamber.