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Effects of carvacrol on sister chromatid exchanges in human lymphocyte cultures

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Abstract

Carvacrol is a predominant aromatic compound in oil of oregano. It has naturally remarkable antibacterial, antiviral, antifungal and antiparasital effects. In this study, genotoxic and antigenotoxic activities of carvacrol were investigated by the *in vitro* sister chromatid exchange (SCE) assay on human peripheral blood lymphocytes.The genotoxicity test was performed with carvacrol in two donors. On the other hand, inhibitory effect of carvacrol was tested in the presence of mitomycin C (MMC) in the same assay. According to data, all doses of carvacrol did not increase the formation of SCE, whereas it inhibited the rate of SCE induced by MMC. In conclusion, carvacrol exhibited a significant antigenotoxic activity in mammalian cells, indicating its potential for use as an antigenotoxic agent.

Abbreviations: BrdU – 5-bromo-2-deoxyuridine; DMSO – dimethyl sulfoxide; GC-MS – gas chromatography-mass spectrometry; MMC – mitomycin C; SCE – Sister chromatid exchange

Introduction

Carvacrol is a terpen constituent of many essential oils extracted from the family Labiatae including Origanum species, and used nowadays on a large scale in the food and cosmetic industries. In addition to its characteristic flavor, many essential oils and especially carvacrol have been shown to exhibit a range of biological activities such as antibacterial, antifungal, insecticidal (Didry et al. 1994; Thompson 1996; Ultee et al. 1998), analgesic (Aydin et al. 1996) and antioxidant (Aeschbach et al. 1994; Puertas-Mejia et al. 2002) activities. It has been shown that carvacrol interacts with the cytoplasmic membrane by its own hydroxyl group and changes the permeability of membrane for protons and potassium ions to exert the antibacterial, antifungal activities (Ultee et al. 2002). On the other hand, it inhibited the production of diarrheal toxin from *Bacillus cereus* (Ultee and Smid, 2001).

Inhibitory effects of carvacrol also have been found in various types of tumorigenesis. Carvacrol inhibited DMBA-induced tumorigenesis in rats and the growth of melanomas *in vitro* (He et al. 1997; Zeytinoglu et al. 1998). Recently, it was reported that Hep-2 cells derived from a human larynx carcinoma show the apoptotic phenotype after carvacrol treatment (Stammatia et al. 1999). In the previous work, we found that carvacrol inhibited the DNA synthesis in normal and ras transformed mouse myoblast cells (Zeytinoglu et al. in press).

Although carvacrol is a common ingredient of the human diet, the increased human exposure to the compound as a result of its application as crop

or food protectant based on their antibacterial and antifungal activity, requires the detection of its genetoxicity. On the other hand, among the naturally occurring antimutagens and anticarcinogens, the antioxidants present in human diet are of great interest (Hartman and Shankel, 1990). Only one report is available on the *in vitro* mutagenicity of the substance (Stammatia et al. 1999) and there is no report on the ability to induce SCE and the antimutagenicity. Therefore, in the present study, the mutagenic and antimutagenic activities of carvacrol were tested for *in vitro* SCE in human peripheral blood lymphocytes.

Materials and methods

Plant extract

Carvacrol (2-methyl-5-(1-methyl ethyl) phenol) examined in this study was isolated from stem distillated essential oil of *Origanum onites* L. collected from West Anatolia (Figure 1). Carvacrol-rich fractions were bulked to obtain carvacrol with 99% purity (GC-MS) (Azcan et al. 2000) and dissolved in DMSO as an 80 μl/ml (v/v) stock.

Sister chromatid exchange analysis

Lymphocyte cultures were set up by adding 0.2 ml of heparinized whole blood from young, non-smoking, healthy one male and one female donor to 2.5 ml of chromosome medium B (Seromed Biochrom). The cells were exposed to 10 μg/ml of 5-bromo-2-deoxyuridine (BrdU, Sigma) at the initiation of cultures and incubated at 37 °C for 72 h in the dark. Test compound was added 24 h after the initiation of cultures. DMSO (20 μg/ml) and mitomycin C (MMC, 1 μg/ml) were used as negative and positive controls, respectively. 0.06 μg/ml colchicine was added to arrest the cells at metaphase 2 h prior to harvesting.

The cells were treated with 0.4% KCl for 25 min at 37 °C, and fixed by cold methanol/glacial acetic acid (3:1) for 20 min. The cells were spreaded on glass slides and then stained with Giemsa according to fluorescence plus Giemsa technique after air-drying (Speit and Haupter, 1985). Fifty well-spread metaphase cells were scanned per concentration (25 cells from each donor); the number of exchanges was scored. The statistical significance for SCE responses was determined by Dunnett test one-way analysis of variances.

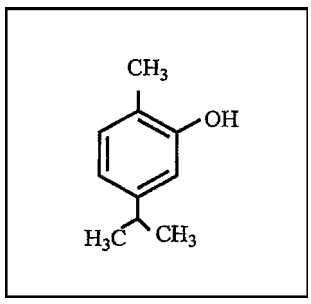

Figure 1. The structural formula of carvacrol (2-methyl-5(1-methylethyl) phenol).

Results and discussion

In order to assess the mutagenic and antimutagenic activity of carvacrol from *Origanum onites* L., SCE was examined in human peripheral lymphocytes. Table 1 shows the summary of the results of SCE assay. No statistically significant increases in SCE were observed at any of the doses tested for carvacrol compared to the controls.

The possible antimutagenic activity of carvacrol was examined towards MMC-induced mutagenesis in the same assay. The results of antimutagenicity testing are given in Table 2. The doses, average number of SCE per cell and the extent of inhibition of mutagenic activity are indicated. Carvacrol strongly reduced the mutagenicity of MMC and dose dependent manner. Linearly increasing dose revealed the inhibition being more pronounced at the lower carvacrol concentrations,

Table 1. Frequencies of sister chromatid exchanges (SCE) in culture human lymphocytes treated with carvacrol

Agent	Doses (μl/ml)(v/v)	Metaphases scored	SCE $\pm$ SE/cell
Control (DMSO)	20.0	50	4.46 ± 0.35
MMC	1.0	50	9.30 ± 0.57
Carvacrol	5.0	50	3.96 ± 0.38
Carvacrol	1.0	50	3.94 ± 0.33
Carvacrol	0.5	50	3.64 ± 0.32
Carvacrol	0.1	50	3.18 ± 0.26

SCE, sister chromatid exchange; SE, standard error; DMSO, dimethyl sulfoxide, solvent control; MMC, mitomycin C, positive control.

Table 2. Antimutagenic effects of carvacrol on sister chromatid exchanges induced by mitomycin C

Agent	Doses (μl/ml) (v/v)	Metaphases scored	SCE ± SE /cell	II %
Control (DMSO)	20.0	50	4.46 ± 0.35	
MMC	1.0	50	9.30 ± 0.57	
Carvacrol + MMC	5.0	50	5.48 ± 0.50*	79.0
Carvacrol + MMC	1.0	50	5.20 ± 0.45*	84.8
Carvacrol + MMC	0.5	50	5.14 ± 0.49*	86.0
Carvacrol + MMC	0.1	50	4.53 ± 0.35*	98.6

DMSO, dimethyl sulfoxide, solvent control; MMC, mitomycin C, positive control; SCE, sister chromatid exchange; SE, standard error; II, inhibitory index.
Dunnett test, $*p < 0.001$.

compared to MMC as a control. The inhibition range was between 79% and 98%. The reason for decreasing inhibition towards higher dose (5 μl/ml) may be due to the slightly genotoxicity of carvacrol as seen in the results of mutagenicity assay (Table 1), although it was not found statistically significant.

Carvacrol as plant volatiles is present in low concentration in human food as a flavor. Toxicological property of carvacrol is becoming important since the use of the compound at higher doses may extend to other application. An oral LD_{50} in rats was calculated for carvacrol as 810 mg per kg body weight (Jenner et al. 1964) and an IC_{50} value in Hep-2 cells was 0.32 mM (Stammati et al. 1999).

Little is known about the genotoxicity of carvacrol examined in the present study. It has been reported that genotoxic potential of carvacrol was very weak both in Ames and DNA-repair test; however it caused DNA fragmentation in Hep-2 cells (Stammati et al. 1999). The weak positive result of Ames test was obtained for only TA 100 strain of Salmonella which was independent of metabolic activation. Antimutagenic activity of carvacrol has not been well studied yet (He et al. 1997; Zeytinoglu et al. 1998).

In our study, results show that carvacrol has a strong antimutagenic effect, supporting its antitumorogenic activities (He et al. 1997; Zeytinoglu et al. 1998). Mechanism of this antimutagenic effect may be due to its antioxidant nature as reported by Aeschbach et al. (1994). Mechanism of antimicrobial effects of carvacrol was suggested to be dependent on its ability to change membrane lipids and permeability of ion channels (Ultee et al. 2002). The antimutagenic activity also might be related to these kinds of effects by inhibiting the uptake of MMC into the cells. Therefore, these must be further substantiated by the *in vitro* antioxidant studies and other relevant assays.

It was confirmed by a set of experiments that at the doses above 10 μl/ml tested did carvacrol show toxic effect against lymphocytes (data not shown), and the decrease in mutagenicity at the lowest dose might be due to its antimutagenic nature. However, since all the assays were performed in the absence of metabolic enzymes such as S9 or P450 which are required for the activation of their metabolites, further studies may be carried out in the presence of metabolic activation to elucidate the effect of carvacrol on promutagens.

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