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Silvia Isabel Rech Franke a b, Temenouga Nikolova Guecheva b, João Antonio Pêgas Henriques c b & Daniel Prá a b
a PPG em Promoção da Saúde, Universidade de Santa Cruz do Sul, Santa Cruz do Sul, RS, Brasil
b Genotox/PPGBCM/PPGBM, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil
c Instituto de Biotecnologia, Universidade de Caxias do Sul, Caxias do Sul, RS, Brasil
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Orange Juice and Cancer Chemoprevention

Silvia Isabel Rech Franke  
PPG em Promoção da Saúde, Universidade de Santa Cruz do Sul, Santa Cruz do Sul, RS, Brasil, and Genotox/PPGBCM/PPGBM, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil

Temenouga Nikolova Guecheva  
Genotox/PPGBCM/PPGBM, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil

João Antonio Pégas Henriques  
Genotox/PPGBCM/PPGBM, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil, and Instituto de Biotecnologia, Universidade de Caxias do Sul, Caxias do Sul, RS, Brasil

Daniel Prá  
PPG em Promoção da Saúde, Universidade de Santa Cruz do Sul, Santa Cruz do Sul, RS, Brasil, and Genotox/PPGBCM/PPGBM, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil

Orange juice (OJ) is among the most consumed fruit juices worldwide, and its chemopreventive action is fairly addressed in the literature. This review critically presents the available evidence linking OJ with cancer chemoprevention and on discussing the putative mechanisms and negative health effects. The chemopreventive action of OJ is related to its effect on metabolic enzymes and negative health effects. Chemopreventive action of OJ is related to its effect on metabolic enzymes and its anti-inflammatory, cytoprotective/apoptotic, hormonal, cell signaling-modulating, antioxidant, and antigenotoxic effects. Most studies on OJ are in vitro, and few are conducted in vivo. Results from in vitro studies must be interpreted carefully because these findings do not consider in vivo bioavailability. However, such results are useful for studying the impact of different processing and storage methods on OJ’s chemopreventive effect. Evidence of OJ’s chemoprevention in humans is limited. OJ is antimutagenic in bacteria and antigenotoxic in humans and rodents. Studies using rodent cancer models showed that OJ is cancer chemopreventive, influencing either the induction stage or the promotion stage. The composition and, therefore, the chemopreventive action of OJ might be influenced by different cultivars, climates, extraction methods, packaging, storage temperatures, and shelf lives, among other factors. Epidemiological studies and randomized controlled intervention studies in humans evaluating the chemopreventive effect of OJ, taking into consideration variability in OJ composition, are needed.

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Address correspondence to Daniel Prá, PPG em Promoção da Saúde, Universidade de Santa Cruz do Sul, UNISC, Av. Independência, 2293, Prédio 42, sala 4206. CEP 96815-900 Santa Cruz do Sul-RS, Brasil. Fax: +5551 3717-1855. E-mail: daniel_pra@yahoo.com

REVIEW SCOPE

In this review, we will present the available evidence of the role of orange juice (OJ) in cancer chemoprevention discussing putative mechanisms involved in this process. Later we will discuss the potential toxicity of OJ and finally, we will critically discuss the available data in terms of evidence-based medicine. Primary studies were retrieved from Pubmed and Web of Science with combinations of the terms: orange juice and oxidative stress, antioxidant, genotoxic, mutagenic, DNA damage, cancer, carcinogenic, anticarcinogenic, anti-inflammatory, immune-modulatory, and cell signaling. Studies were manually selected to be included in the review. Additional studies were selected as references from the cited papers. Only studies using commercial or OJ prepared by the researchers were included in this review. Studies based on extracts from oranges were avoided and only mentioned to corroborate primary evidence provided by results with OJ.

OJ

Oranges are among the most consumed fruits worldwide, which reflects the fact they have been cultivated since ancient times. They are widely grown in warm climates worldwide; Brazil and the United States produce about 50% of the total world supply. The flavors of oranges vary from sweet to sour. The fruit is commonly peeled and eaten fresh or squeezed for its juice. There are 2 kinds of orange; Citrus sinensis is called sweet orange and Citrus aurantium is called sour orange (other names include bitter orange, bigarade orange, and Seville orange) (1). Sweet oranges can be further divided into 2 varieties, blond and blood oranges, based in their pulp coloration. Blond oranges...
have a smooth peel, soft pulp, weakly winged leafstalk (2) and regular-colored juice. Blood (red pulp) oranges are richer in anthocyanins and produce darker juices (3). OJ is among the most consumed fruit juices and OJ itself and its constituents may exert various biological effects (4–7). OJ is considered among the top micronutrient-dense juices (i.e., a food that provides substantial amounts of vitamins and minerals and relatively fewer calories) (8). Recent evidence from a study comparing adults over 19 years of age (n = 8,861), who were consumers and nonconsumers of OJ participating in the National Health and Nutrition Examination Survey, 2003–2006, have shown that consumption of OJ (usual per capita intake of 100% OJ was 50.3 mL/day) was associated with better diet quality and an increased prevalence of meeting the estimated average requirement for key nutrients (mainly vitamin C and folate) as well as other biomarkers of positive health outcomes, including lower total cholesterol and LDL levels. The study also indicated that consumers of OJ had lower mean body mass index and a decreased risk of obesity (9).

OJ can provide substantial amounts of vitamin C and folic acid, 2 well-known substances that can reduce DNA damage levels and, thus, the risk of cancer (10, 11). OJ also provides substantial amounts of phenolic substances, particularly of flavonoids, that can also have chemopreventive action (12). The effect of these substances will be addressed later in this review.

The composition of oranges and, therefore, OJ, varies depending on varietal, climatic conditions, soil composition, light and pathogen exposure, and maturation stage during harvesting period, as well as on storage and post-harvest processing (13).

Thus, it is important to highlight that the reviewed evidence will depend on the experimental designs, the purpose of the study, the doses of OJ administered, the treatment scheme, as well as the system or subsystem (e.g., cell culture versus human populations) under study; therefore indicating extensive differences among the studies.

**OJ AND CANCER CHEMOPREVENTION**

Table 1 summarizes epidemiological and murine cancer model studies with OJ. Primary epidemiological evidence is inexistent for OJ. The few studies available indicated that OJ was not associated with a reduced risk of squamous cell carcinoma of the skin (14) and there is evidence that it might increase the risk of melanoma (15). Evidence also indicates OJ can reduce the risk of leukemia in children (16).

Conversely, the murine models of cancer (Table 1, bottom) have shown that OJ can be chemoprotective against mammary (17), hepatic (18), and colon cancer (19). For mammary carcinogenesis, female Sprague-Dawley were treated with 5 mg dimethylbenz[a]anthracene, as a single intragastrically dose at approximately 50 days of age (while in diestrus), and double-strength OJ was administered ad libitum (108.5 mL, per rat per day) for the next 15 wk. OJ was able to reduce the mammary tumor weight and tumor burden (17). This effect seemed to be mediated by the synergistic action of hesperetin with other compounds that have anticancer activity (20). In hepatic carcinogenesis chemoprotection, Fischer 344 male rats were treated daily with 250 μg/kg aflatoxin B-1 after reaching 85 g, with 10 alternate doses, and with OJ extract (0.5 mg/kg) either concomitantly with aflatoxin B-1 treatment (induction) or every 2 days after the induction for 12 wk (promotion). OJ extract substantially decreased the number of gamma-glutamyl transpeptidase-positive foci in liver when administered during the initiation period, whereas during the promotion period caused a decrease in the average diameter of the foci. The total volume of foci was markedly reduced by OJ during either period (18). Therefore, OJ extract treatment in this murine models of cancer seemed to inhibit the biochemical and cellular events associated to either initiation or promotion (18). In colon carcinogenesis chemoprevention experiments, male Fischer 344 rats received 15 mg/kg azoxymethane by subcutaneous injections at 22 and 29 days of age and 1 wk later pasteurized OJ in place of drinking water for another 28 wk. At the end of the experiments, colon tumor incidence and proliferating cell nuclear antigen-positive nuclei in colon crypts was reduced, and there was a strong trend toward a smaller average tumor burden (mg tumor/rat) for the group drinking OJ (19). This results showed an inhibition of clonal expansion of transformed cells into visible polyps or tumors when drinking water was replaced by pasteurized OJ (19).

**CHEMOPROTECTIVE MECHANISMS OF OJ**

Figure 1 summarizes the chemopreventive mechanisms of OJ, including antioxidant, antigenotoxic, cytoprotective/apoptotic, antiinflammatory, cell-signaling, phytoestrogen, antimicrobial, and antiviral effects as well as an effect on metabolism and excretion of xenobiotics. These effects are detailed in the following sections.

**Antioxidant Potential of OJ**

There are many studies evaluating the antioxidant potential of OJ, mostly in vitro. All in vitro studies have shown that OJ has considerable antioxidant potential. The high content of flavonones is linked to OJ’s antioxidant potential. Juices rich in flavonones are the second-best antioxidant, after fruits rich in anthocyanins (red, purple, or blue fruits) (21–23).

Certain factors affect in vitro antioxidant potential. High temperatures during thermal treatment (3, 24, 25) or during storage (≥20°C) and long-term storage (≥4 mo) (4, 25–27) decrease antioxidant activity due to phytochemical composition changes (3, 27, 28). Sweetening seems to lead to the extinction of most antioxidant activity (4).

To date, it is not clear how much each OJ constituent contributes to the overall antioxidant activity. It is possible that some compound act at short-term losing their antioxidant capacity (e.g., vitamin C) and other such as phenolics might retain their antioxidant potential for longer periods (e.g., phenols are stable for long periods but change in term of their relative components) (29, 30). The test system used to evaluate it, which


## Table 1

Summary of the epidemiological and murine models studies evaluating orange juice (OJ) chemoprevention

<table>
<thead>
<tr>
<th>Food</th>
<th>Type of study/endpoint</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>OJ, oranges, or citrus peel</td>
<td>Population-based case-control study evaluating the relationship between citrus consumption and the history of squamous cell carcinoma of the skin in an elder Southwestern U.S. population (n = 242 cases and 228 controls)</td>
<td>Consumption of OJ [OR = 1.00 (0.83–1.20)] or [OR = 0.89 (0.71–1.11)] were not associated to the reduction of skin carcinoma risk, but citrus peel intake reduced its risk [OR = 0.66 (0.45–0.95)].</td>
<td>(14)</td>
</tr>
<tr>
<td>OJ</td>
<td>Follow-up study in 2 Nurse’s Health Study cohorts evaluating the relationship between vitamins and certain foods and the risk of melanoma (n = 162,000)</td>
<td>The frequency of OJ consumption was linearly associated with increasing relative risk of melanoma (P &lt; 0.01)</td>
<td>(15)</td>
</tr>
<tr>
<td>OJ</td>
<td>Case-control study aiming to study the relation between child’s early diet and risk of childhood leukemia in diverse California population (n = 328 case-control sets)</td>
<td>OJ regular consumption during the first 2 years of life was associated with a reduction in risk of childhood leukemia diagnosed between the ages of 2 and 14 yr [OR = 0.54 (0.31, 0.94)]</td>
<td>(16)</td>
</tr>
<tr>
<td>Double-strength OJ</td>
<td>Evaluation of mammary tumors induced by 7,12-dimethylbenz[a]anthracene in female Sprague-Dawley rats</td>
<td>Mammary tumor development was delayed and tumor burden (grams of tumor/rat) was reduced</td>
<td>(17, 20)</td>
</tr>
<tr>
<td>OJa</td>
<td>Evaluation of preneoplastic foci in the liver of Fischer 344 male rats treated with aflatoxin B-1</td>
<td>Decrease in number (initiation) and volume (promotion) of gamma-glutamyl transpeptidase-positive foci in liver</td>
<td>(18)</td>
</tr>
<tr>
<td>Pasteurized OJ in place of drinking water</td>
<td>Evaluation of colon cancer in male Fischer 344 rats treated with azoxymethane</td>
<td>Colon tumor incidence was reduced by 22%</td>
<td>(19)</td>
</tr>
</tbody>
</table>

**OR = odds ratio with 95% confidence interval (in parenthesis).**

*aAn extract of orange juice (OJ) was used to treat the rats.

might help in interpreting their real beneficial effect in vivo, can also influence the results. Results from in vitro studies must be interpreted carefully because these findings do not consider in vivo bioavailability.

Most studies evaluating the antioxidant effects of OJ in humans and rats showed antioxidant effects. A single intake of OJ can increase the blood radical scavenging capacity from 90 min (150 mL) (31) to 1 day (300 mL) (32). A single intake of OJ (600 mL) also increases the antioxidant capacity for more than 3 h (33). Two-week treatments of healthy volunteers (up to 500 mL OJ daily) reduced the levels of lipid peroxidation (34). Dosages equal or greater than 600 mL daily for more than 2 wk have been shown to induce not only a short lipid peroxidation decrease but a reduction in DNA oxidation as well (6, 35). Processing and storage can have little or no influence or completely abolish antioxidant effects (32), but further studies are still needed to better understand this issue. In vivo data also confirm the observation that storage reduces the antioxidant effect of OJ, particularly due to the loss of vitamin C (32). Results of OJ administration in rats confirmed the effect in humans, indicating that longer administrations (6 mo) of daily doses comparable to humans (1 mL for a rat equals 600 mL for a human) might increase antioxidant capacity in the long term (36). It is important to mention that the extrapolation of murine data on OJ administration to effects in humans must be done with care. Although rodents generally have extensive biological homology to humans, their ability to synthesize vitamin C (37) makes them less favorable models for OJ testing. There is a lack of studies evaluating the impact of factors that impair the in vitro antioxidant effect with the in vivo antioxidant effect.

### Antimutagenic and Antigenotoxic Effects of OJ In Vivo

OJ reduced the mutagenicity induced by different mutagens as evaluated by the Salmonella/microsome assay (Ames test) with and without metabolic activation. OJ was more effective as cotreatment and was most potent inhibitor in relation to other substances tested (38–40).

Riso et al. (35) showed that lymphocytes of individuals supplemented with blood OJ (600 mL × 21 days) had increased resistance to H₂O₂-mediated DNA damage, as evaluated by...
FIG. 1. Activities and effects of orange juice on the multistage process of carcinogenesis.

The comet assay. In mice, our data indicate that OJ (a single dose equivalent to 600 mL in humans) reduced the genotoxicity induced by alkylating agents, iron, and copper, also using the comet assay. Differences were observed depending on the treatment schedule (OJ as pre- or posttreatment). OJ, tested either before or after treatment with the alkylating agent methyl methanesulfonate, reduced the DNA damage (by approximately 70% as a pretreatment and by approximately 40% as a posttreatment). The pretreatment reduced the genotoxicity of either iron or copper by approximately 50%. No significant effect was seen during posttreatment with OJ after treatment with iron and copper (41, 42). Guarnieri et al. (43) evaluated the effect of the intake of a single portion of blood OJ (300 mL) on mononuclear blood cell resistance to H2O2-induced DNA damage (43) by the comet assay.

The comet assay detects DNA single-strand breaks, alkallable sites, DNA-DNA/DNA-protein crosslinking, and single-strand breaks associated with incomplete excision repair sites (44). OJ could therefore reduce these types of DNA damage.

In support of an antimutagenic effect of OJ, a reduction in the frequency of micronuclei was also detected in mice exposed to radiation or cyclophosphamide and treated with orange extracts (45, 46). Oshawa et al. (47) found no suppression of primary DNA damage in the liver or stomach of mice orally treated with OJ just before a simultaneous oral dose of morpholine and NaNO2. Such results indicate the DNA-protective effects of OJ might depend on the nature of the DNA damage generated.

Cytoprotective/Apoptotic, Antiinflammatory, and Cell Signaling Effects of OJ

There is extensive data supporting the role of inflammation in cancer, because many cancers arise from sites of infection, chronic irritation, and inflammation (48). The antiinflammatory action of plant phytochemicals has been advocated as an important mechanism of reducing cancer risk (12). In spite of many studies addressing the effect of OJ constituents individually, fewer studies address the effect of the whole OJ in tissues. OJ significantly reduced plasma concentrations of F2-isoprostanes...
and might also reduce C-reactive (34, 49) and markers of oxidative stress and inflammation, which are typically increased in the plasma in prooxidative states such as diabetes (33).

In vitro, OJ has differential effects on cell survival. OJ was shown to increase metabolism and proliferation of cells. Ekmekecioglu et al. (50) evaluated the effect of OJ on the intestinal cell line Caco-2 in terms of toxicity, growth and differentiation. Cells exposed to fresh OJ (50% v/v) exhibited higher tetrazolium reduction rates in the MTT assay (121.3% of control). These cells also showed higher succinate-cytochrome c reductase activities than the other samples, implying that the contents of fresh OJ, such as ascorbic acid, stimulated mitochondrial metabolism (50). Lim and Lim (51) also found 10% and 30% but not 50% (v/v) OJ could increase Caco-2 cell viability by up to 50% in relation to controls. On the other hand, extracts from blood OJ were shown to inhibit proliferation of lung fibroblasts and epithelial prostate cancer (52).

There are, to date, no studies on the effects of OJ on cell signaling in cancer chemoprevention, but evidence of a cell-signaling role for individual OJ constituents in cancer chemoprevention is accumulating. For instance, both hesperadin (53) and vitamin C (54) have been shown to induce apoptosis, possibly by different mechanisms. On the other hand, there is also growing evidence that vitamin C (100 μM) protects skin cells by promoting fibroblast proliferation, migration, and replication-associated base-excision repair of potentially mutagenic DNA lesions (55). Other compounds such as limonoids are also potent antiproliferative agents in different cancer cells (56).

**Phytoestrogen Effect of OJ**

Numerous epidemiological studies suggest that diets rich in phytoestrogens may be associated with low risk of some cancers, especially steroid hormone-dependent cancers (e.g., breast and prostate) (57). OJ contains low to moderate amounts of phytoestrogens (58), and hesperadin, one of the major bioactive components of OJ, has been prescribed for the treatment of hot flashes associated with menopause (59). Naringenin has also been shown to significantly increase uterine weight in rats through a tissue-specific effect on estrogen receptor-α distribution. Possibly because of its flavonoids, OJ can also improve bone health in orchidectomized old rats with osteoporosis, also though a hormone-linked pathway (60).

**Antimicrobial and Antiviral Effects of OJ**

There is growing evidence of the impact of viral and bacterial infections on cancer risk (61). Although there is evidence of the antimicrobial and antiviral effects of OJ, these aspects are less studied. It is generally believed that OJ can prevent common colds, although the only available robust evidence is that OJ (62) or vitamin C (63) can reduce the severity and duration of common cold-associated symptoms. Moreover, OJ could provide a physiological level of ascorbic acid, similar to those levels achieved by taking high doses of ascorbic acid (62). OJ, similar to other juices, has been shown to inhibit certain types of infections. The inhibition of bacterial adherence to bladder cells has been assumed to account for beneficial effects on the prevention of urinary tract infections. Zafriri et al. (64) showed that commercial OJ inhibits the adherence of Escherichia coli to eukaryotic cells.

**Effects of OJ on Bioavailability of Nutrients, Medicines, and Xenobiotics**

OJ has been shown to enhance the absorption of minerals such as iron (65), aluminum (66), calcium (67), zinc (68), and selenium (69). OJ was also shown to modulate the absorption of certain amino acids. There is evidence that OJ increases the absorption of glycine but not lysine and methionine (70).

OJ has also been shown to interfere with the bioavailability of several medicines. OJ was shown to reduce the absorption of acetylsalicylic acid (71), β-adrenergic blocking agents (72, 73), antihistamines (74), HIV-protease inhibitors (75), chemotherapeutic agents (76), and hypoglycemic drugs (77). Because of the potential influence of OJ on drug absorption and metabolism, it is recommended that patients avoid consuming OJ while taking medication and that healthcare providers advise against OJ intake until any interactions with specific drugs can be clarified in clinical studies (78).

OJ might influence the bioavailability of substances by different mechanisms, including pH changes in the intestinal lumen, chelation, and interference with phase I and II enzymes. OJ’s acidity, influence on gastric emptying (71), influence on metabolic enzymes (72), and/or influence on the influx or efflux of compounds from the interior of cells to the extracellular space (74, 76, 79) can either stimulate or reduce drug absorption. OJ can therefore affect the bioavailability of drugs, bioactive food ingredients, and/or foodborne toxic compounds upon oral uptake (79).

**Putative Compounds in OJ Cancer Chemoprevention**

The subject of which component of OJ is more effective in cancer chemoprevention is controversial (20, 80). Of these, the flavonoids are the most studied. The antiproliferative effects of OJ flavonoids are possibly due to regulation of the cell cycle (17, 20, 56), among other effects. Flavonoids have been shown to regulate the downstream genes that are responsive to the nuclear factor of kappa light-chain enhancer of activated B cells (NFκB) and mitogen-activated protein kinase (MAPK) signaling cascades. NFκB involves a transcription control mechanism of cellular responses to stimuli such as oxidative stress, inflammation and bacterial and viral infections that are associated with cancer (81). MAPK is an enzymatic system that responds to extracellular mitogenic stimuli and regulates various cellular activities, such as cell division and proliferation, differentiation and apoptosis (82, 83).

Heperidin and naringin and their aglycones, hesperitin and naringenin, respectively, are the most abundant and studies flavonoids of OJ. Pharmacokinetic studies indicate that ingestion of OJ and other citrus fruits and juices may give rise to
tissue levels of flavonoids sufficient to exert a biological effect in humans, on the order of 0.5 μM naringenin in plasma and 10 μM naringenin in liver. In agreement with this, an ingestion of 400–760 mL OJ was shown to result in 0.6 μM naringenin in serum (84).

Hesperidin 25 mg/kg body weight was shown to protect lung carcinogenesis in mice (85) and, at 1000 ppm in the diet, was shown to reduce rat oral (86, 87), esophageal (88), urinary-bladder (89), and colon (90) carcinogenesis. There is evidence of an anticancer effect of hesperidin in skin tumors in mice, mainly in the promotion stage but not in cancer initiation (91, 92), and much of its effect has been associated with the inhibition of cell cycle progression (89). Hesperidin has been used as a pharmaceutical because of its ability to improve the permeability and integrity of the capillary lining (93). Hesperidin action (200 mg/kg oral administration) in acute lung injury involves the suppression of inflammation, as seen by the downregulation of many inflammatory interleukins, chemokines, adhesion molecules, and nitric oxide and leukocyte infiltration, as well as the inhibition of the phosphorylation of IkB, a blocker of NFκB, p38MAPK and JNKMAPK (94). Hesperidin (10 and 25 mg/kg) was shown to ameliorate colonic inflammation by reducing colonic damage and colonic mieloperoxidase activity in animal models of inflammatory colitis (95). Hesperidin (5–100 μM in a dose-dependent fashion) was shown to inhibit mitogenic stimulation of aortic vascular smooth cells by arresting the cells with no apoptosis. Cell cycle arrest was associated with an upregulation of p27kip1 in parallel with the downregulation of retinoblastoma protein, cyclins, and proliferating cell nuclear antigen, but there were no changes in MAPK (96). Hesperidin (10 and 100 μM in a concentration-dependent fashion) also seems to induce cytotoxicity of colon cancer cells through the downregulation of B-cell CLL/lymphoma 2 (BCL2) and upregulation of caspase-3 and Bcl-2–associated X protein (Bax) (97). Hesperidin in vitro (50–200 μM) and in vivo (25 mg/kg) was shown to contribute against dysplasia by inhibiting cell hepatocytes invasion and downregulating metalloproteinase expression, a family of enzymes involved in extracellular matrix degradation (98, 99).

Similarly, naringenin in vitro (0.01–0.3 μM) and in vivo (50 and 100 mg/kg, in a dose-dependent manner) has been shown to induce antiinflammatory effects in different cells (100, 101). Both naringenin and hesperidin (0.01–0.3 μM) inhibited TNF-α production at similar levels in glial cells, but naringenin inhibited iNOS in a concentration-dependent fashion, whereas hesperidin was not effective in inhibiting the enzyme. Naringenin but not hesperidin was also found to reduce cell mortality and inhibit the phosphorylation of p38MAPK and STAT-1α (a member of the signal transducers and activator of transcription family, which have a role in immune maturation and tolerance as well as in tumor surveillance). The exclusive feature of naringenin regarding the presence of the hydroxyl group in the B-ring instead of a catechol, as in all other flavonoids, might explain this activity (100). Naringin and naringenin (10–50 μM), conversely to other flavanones, were not capable of reducing viability, invasion, motility, and cell-matrix adhesion in human lung adenocarcinoma cells (102). Interestingly, a bitter orange extract rich in naringin and neohesperidin was shown to repress plasminogen activator inhibitor 1 (PAI-1), a serine protease inhibitor, and to upregulate matrix metalloproteinase 12, a macrophage elastase, in human colon fibroblasts treated with TNF-α but not in controls. This suggests different effects on healthy and diseased cells, which may be beneficial in healthy cells to prevent sustained inflammation (93).

Beyond flavonoids, OJ has been used as a source of various micronutrients and phytochemicals with or without the addition of vitamins and other compounds as supplements (103, 104). Therefore, the many other constituents of OJ should not be overlooked.

Limonoids are strong antioxidants (105) that have antiproliferative effects (56). Recent studies have indicated that D-limonene is a potential chemotherapeutic agent, but this drug’s mechanism remains to be elucidated. The available evidence indicates that D-limonene induces apoptosis via the mitochondrial death pathway and the suppression of the PI3K/Akt pathway (106). D-limonene also seems to block angiogenesis by inhibiting vascular endothelial growth factor and to block metastasis by reducing the expression of matrix metalloproteinases (107).

Coumarins (108) and bioactive amines (7) are also present in OJ. Beyond studies of the antioxidant properties of certain coumarins (109) and bioactive amines (110), the results of further studies regarding the chemopreventive actions of OJ-specific compounds are still pending.

The vitamin C content of 500 mL OJ is approximately 250 mg (111), which can lead to approximately 70 μM vitamin C in the serum (112). Vitamin C protects against the occurrence of several types of cancer, including mouth, esophageal, gastric, pancreatic, and rectal cancer (113–115).

Another compound for which OJ is a key biological source is folate. For instance, 500 mL OJ can provide 150 μg folate (111), nearly 40% of the Dietary Reference Intake for the nutrient (116). OJ is among the major food sources of folate on a given day for the U.S. population (117). Folate is involved in DNA synthesis, repair, and methylation (11). Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage (118). The folate status modulates the risk of developing cancers in selected tissues, the most notable of which is the colorectum (119).

**POTENTIAL TOXICITY OF OJ**

**General Effects**

OJ intake can generate noxious effects for some individuals, particularly in large amounts. For example, excessive amounts of OJ might induce hyperkalemia, especially in renally compromised individuals (120). OJ has been controversially linked to the risk of food allergy in sensitive individuals (121, 122). OJ was found to stimulate salivary secretion, decrease the salivary pH immediately after consumption, and decrease the redox
potential of whole saliva. This can be caries-promoting in the oral cavity in persons with low salivary flow rate who consume the juice regularly (123). Moreover, sour (Seville) orange (Citrus aurantium) extracts have been shown to induce weight gain and increase blood pressure and cardiac dysfunctions in hypertensive subjects, possibly because of its synephrine, octopamine, and furocumarin content. Indeed, sour orange juice (SOJ) should be avoided by individuals with severe hypertension or glaucoma and by those taking monoamine oxidase inhibitors. SOJ consumption seems to be safe for normotensive subjects (124).

OJ should be considered as a part of the recommended daily ingestion of at least five portions of fruit and vegetables to prevent cancer in healthy adults (125). However, the recommended daily portions for intake of fruit juices among children and hypertensive, kidney-compromised, or diabetic subjects could be lower than recommended for healthy people. The use of OJ as a substitute for meals can lead to malnutrition and decreased stature in children (126). Excessive intake of any food, even for the healthiest, can lead to oxidative status imbalance.

Unpasteurized OJ is a known vehicle for salmonellosis, and several cases of outbreaks of bacterial infections were reported in the United States (127).

Prooxidant Effect, Genotoxicity, and Carcinogenicity of OJ: Is There Any Harm?

Despite scientific data reporting beneficial properties derived from the consumption of OJ (for example, antmutagenic or anticarcinogenic effects), some compounds also present in OJ have been identified as being mutagenic or carcinogenic (5, 42, 128). The carcinogenic or genotoxic effects of OJ might be mediated by the interaction of juice components with transition metals or by sub-products of juice autoxidation. Some in vitro and in vivo studies report prooxidant and genotoxic effects for OJ. For example, Franke et al. (4) showed significant increases in lipid peroxidation induced by OJ in vitro. In vivo results also showed an increase of lipid peroxidation in the serum of healthy volunteers 2 h after the intake of OJ but only for 8-day-aged juice stored at 4°C (32).

There is evidence of OJ mutagenicity in bacteria. Mazaki et al. (129) observed mutagenic activity in Salmonella/microsome assay. Heated OJ, as well as the acid hydrolysate, were mutagenic and cytotoxic in Salmonella typhimurium without S-9-mix (hepatic microsome fraction, added to simulate mammalian metabolism), after neutralization to pH 7.4 (130, 131). More recent data of Friedman et al. (132) did not confirm the dramatic increase in mutagenicity reported for heated OJ, and the response without S-9 activation was similar for juices either heated or not, ranging from 2 to 3 times the background values. In agreement with the previous results, Franke et al. (4) evaluated the mutagenic activity of frozen and fresh forms of in natura and processed OJ (pH not adjusted) using 4 strains of Salmonella in the Ames test with or without S-9-mix. Only 1 unsweetened, unfrozen, and processed OJ sample was not mutagenic for any of the strains tested. This OJ also had the highest antioxidant activity. Interestingly, the content of vitamin C and phenolic compounds correlated to the mutagenicity (4). Although heating, normally used in processing OJ, is insufficient to release mutagenic flavonol as aglycones from their glycosides (kaempferol and quercetin), other compounds such as Maillard intermediary product (130) or mutagenic browning products derived from free lysine, histidine, or other amino acids (132) might be generated. As an alternative hypothesis to OJ mutagenicity, one should recall that plant-derived compounds have antibacterial activity (133), which might partially explain the OJ mutagenicity in bacteria.

In mammals there is no evidence of the genotoxicity of OJ. Nevertheless, higher doses of OJ (equivalent to ~600 mL in humans) can induce a slight transient increase in DNA damage in blood cells of mice, as detected by Franke et al. (41, 42). Evidence linking OJ consumption to cancer risk is sparse and controversial. It has been recently hypothesized that cutaneous melanoma grows as the availability and consumption of citrus products increases, which may be related to concomitant increases in dietary photocarcinogenic furocoumarins (134). In support of this hypothesis, Feskanich et al. (15), studying 2 Nurse’s Health Study Cohorts, found an unexpected association between the increased risk of melanoma and higher intake of food vitamin C, particularly from OJ. The authors describe this association as random but also state that the association was strongest among the higher-risk, sun-sensitive women. It is unlikely that vitamin C was responsible for the association, because supplements of vitamin C were not associated with a higher risk of melanoma. OJ contains appreciable quantities of furocoumarins such as psoralens. Psoralens are substances that cause interstrand cross-links and monoadditions in DNA when photoactivated by UVA (135). On the contrary, the case-control study of Hakim et al (14) with elder from Arizona indicated that the intake of citrus peel reduced the risk of squamous cell carcinoma of the skin, pointing out an intake-dependent chemopreventive effect of substances present in citrus peel such as d-limonene. Considering this data, epidemiological studies evaluating either the beneficial or noxious effect of fruit juices are needed, particularly for those taking into account differences in the processing and composition of these drinks, as well as controlling for confounding variables, such as lifestyle and diet, that may lead to false interpretations.

WEIGHT OF EVIDENCE REGARDING CANCER CHEMOPREVENTION BY OJ

To date, no epidemiological study specifically evaluated the chemopreventive action of OJ in humans. There are few studies using rodent models of cancer that evaluated the effect of OJ on cancer chemoprevention. The results from the rodent cancer models revealed relevant cancer chemoprevention evidence for OJ according to the “hierarchy of robustness,” as defined by the Joint Panel of the World Cancer Research Fund and the American Institute for Cancer Research (136) for evaluating cancer
chemoprevention. The Panel ranks human and animal experimental studies in evaluating the role of dietary and physical activity in the risk/prevention of human cancer. Class 1 evidence refers to 1) in vivo data from controlled human feeding studies, 2) data from genetically modified models of human diseases, and 3) in vivo studies using rodent cancer models designed to investigate modifiers of the cancer process (136). The available evidence can be classified as Class 1c because of the studies using rodent models of cancer, summarized in Table 1.

Several biological effects that can contribute to chemoprevention were shown for OJ. In this review, we summarized several of these effects, including antioxidant, antimutagenic and antigenotoxic, cytoprotective, hormonal, and cell signaling-modulating effects. OJ has antimicrobial and antiviral action and modulates the absorption of xenobiotics. Therefore, OJ could contribute to chemoprevention at every stage of cancer initiation and progression. Among the most relevant biological effects of OJ is the juice’s antigenotoxic and antimutagenic potential, which was shown in cells in culture and in rodents and humans.

The biological effects of OJ in vitro were shown to be largely influenced by the juice’s composition. The composition of OJ depends on physiological conditions (related to climate, soil and fruit maturation, the genetic characteristics (varietal) of the oranges and variations in processing methods and storage times and methods. The addition of sugars seems to substantially decrease the antioxidant effect of OJ. Thermal treatments, storage above 20°C or both can lead to an even greater decrease in antioxidant activity.

There are numerous studies evaluating the biological effects of the constituents of OJ. Although many of these components are chemopreventive agents in isolation, it is difficult to understand how the compounds interact when ingested as part of OJ. It is very likely that the individual constituents of OJ act synergistically and antagonistically rather than simply by an additive mechanism.

Epidemiological studies and randomized controlled intervention studies in humans evaluating the chemopreventive effect of OJ, in terms of amount, consistency, and quality, on cancer risk are needed to establish this juice’s chemopreventive effect. The type and amount of OJ to be tested should be defined with caution, as excessive intake of OJ can be noxious to children (cavities and undernutrition may occur if OJ is used as a main meal substitute) or hypertensive (in increases in sour OJ consumption could lead to a pressure increase and cardiac dysfunction), renally compromised (hyperkalemic), or diabetic (hyperglycemic) subjects. OJ should be also pasteurized given the risk of bacterial outbreaks.

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REFERENCES

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