



REVIEW ARTICLE

Potential Cancer Chemopreventive Activity of Protocatechuic Acid

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A natural phenolic compound, protocatechuic acid (3,4-dihydroxybenzoic acid), is present in many edible and medicinal plants. Recent studies, including our animal experiments, indicate that this simple phenolic acid could be protective against the development of epithelial malignancy in different tissues and cardiovascular diseases as well. The mechanism of the action is mostly associated with antioxidant activity, including inhibition of generation as well as scavenging of free radicals and upregulating antioxidant enzymes. The influence on Phases I and II of the metabolism of certain carcinogens and, perhaps, direct blocking of specific binding sites of ultimate carcinogens with DNA molecule, thus preventing adduct formation that may result in mutations and neoplastic transformation, also account for its cancer protective action. However, other biological aspects of the chemopreventive activity of protocatechuic acid are not fully studied. They include influence on the activity of inducible isoenzyme of cyclooxygenase and nitric oxide synthase, cell cycle-regulating proteins, or inflammatory cytokines, which are involved in oncogenesis. In view of its reported biological properties and relative safety, protocatechuic acid is a potential cancer chemopreventive product.

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1. Introduction

Prevention of chronic diseases, including cancer, is an old but important concept regarding human health. Dietary factors are known to influence cancer development.^{1,2} An important consideration in cancer research today is that exposure to pharmacologically active chemicals (natural and synthetic) may play an important role in reducing the risk of cancer development.^{1,3,4} Cancer chemoprevention could be possible by the use of exogenous factors to enhance endogenous mechanisms that reduce the risk of cancer development because of exposure to different environmental factors. Some of these exogenous factors are dietary constituents, drugs, immunizations, and supplements. Edible plants and plants used for folk medicine are rich sources of such cancer chemopreventive agents.^{3–6}

Protocatechuic acid (3,4-dihydroxybenzoic acid; Figure 1) is a simple phenolic compound widely distributed in nature. Like many other simple phenolic acids, protocatechuic acid is detected in almost all plants and is, therefore, a very common component of human diet,⁷ such as the bran and grain brown rice (*Oryza sativa* L.)⁸ and onion (*Allium cepa* L.),⁹ especially in the scales. Protocatechuic acid is detected in many fruits, such as plums (*Prunus domestica* L.)¹⁰;

gooseberries (*Ribes uva-crispa* L.)⁹; grapes (*Vitis vinifera*)¹¹; and nuts, such as almonds ordinary (*Prunus amygdalus*).¹² It is present in products of plant origin, such as olive oil or white wine.^{13–15} Protocatechuic acid is also found in many plants and spices, such as star anise (*Illicium verum*), melisse (*Melissa officinalis* L.), a medical rosemary (*Rosmarinus officinalis* L.), and cinnamon (*Cinnamomum aromaticum*).⁹ This compound is one of the biologically active components of some medicinal plants, including those used in natural medicine, such as sudan Mallow (*Hibiscus sabdariffa* L.),^{16,17} Japanese ginkgo (*Ginkgo biloba* L.),¹⁸ and St. John's wort (*Hypericum perforatum* L.).¹⁹

We demonstrated the chemopreventive ability of protocatechuic acid in chemically induced carcinogenesis in mainly the digestive organs of experimental animals.^{20,21} In this review, we have highlighted the protective mechanisms of protocatechuic acid against carcinogenesis.

2. Effects of Protocatechuic Acid on Chemical Carcinogenesis in Rodents

The chemopreventive action of protocatechuic acid was evaluated in several models of chemically induced carcinogenesis in laboratory animals (Table 1).^{16,22–33} The results indicate that the protocatechuic acid at doses of 200–2000 ppm in diet effectively inhibited the development of most of the cancers, especially of the digestive

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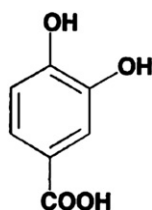


Figure 1 Chemical structure of protocatechuic acid (3,4-dihydroxybenzoic acid). Chemical abstracts service (CAS) No.: 99-50-3; Molecular weight (MW): 154.12.

system,²¹ when administered both in the initiation phase and in the promotion/progression of carcinogenesis.

Efficacy of protocatechuic acid was demonstrated in the prevention of cancers of the oral cavity in several experimental models using rats and hamsters. Dietary feeding with protocatechuic acid during the initiation phase or the promotion/progression of tongue carcinogenesis of rats reduced the incidence and the number of preneoplastic lesions (hyperplasia and dysplasia) and epithelial neoplasms (squamous cell carcinomas and papillomas) induced by 4-nitroquinoline oxide.²² A recent study with modified experimental protocol using the same carcinogen, which was able to cause a greater percentage of carcinomas in the advanced stage, has shown that treatment with protocatechuic acid in the progression phase reduced the incidence of precancerous lesions and cancers invading adjacent organs or metastasizing to the lungs.²³ Protocatechuic acid given during the promotion/progression of carcinogenesis induced by 7,12-dimethylbenz[*a*]anthracene in the cheek pouch of hamsters significantly reduced the size of tumors and the area occupied by a precancerous lesion, squamous cell dysplasia.²⁴

In the model of rat colon carcinogenesis induced by azoxymethane, protocatechuic acid in diet during the initiation or promotion/progression lowered the number of aberrant crypt foci,

which are considered to be putative precancerous lesions,^{34–36} and the incidence and number of colorectal adenocarcinoma.^{5,25,34}

Protocatechuic acid administered during promotion/progression of pancreatic carcinogenesis induced by *N*-nitrosobis(2-oxopropyl)amine in Syrian golden hamsters caused a significant reduction in the incidence of large pancreatic cancer (>3 cm) invading the adjacent organs.²⁶

Protocatechuic acid also affected the development of neoplasia in the rat liver induced by diethylnitrosamine.²⁷ When protocatechuic acid was administered in diet during the initiation or promotion/progression phases of carcinogenesis, the incidence of altered hepatocellular foci, characterized by lack of iron accumulation and being positive for the reactivity against placental isoform of glutathione S-transferase (GST), was decreased. The treatment also reduced the multiplicities of both liver cell adenomas and carcinomas. Chemoprevention effects of protocatechuic acid in urinary bladder carcinogenesis was also revealed in rats initiated with *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine by the reduction of the incidence of precancerous lesions (hyperplasia and dysplasia) and cancers when rats were fed the diet containing the chemical during the initiation or promotion/progression stages.²⁸

Our data on cancer chemopreventive ability of protocatechuic acid indicate that dietary administration with protocatechuic acid at 500 or 1000 ppm during the initiation and postinitiation stages suppresses chemically induced carcinogenesis in the tongue, glandular stomach, colon, liver, and urinary bladder of rats,^{21,28} suggesting that 500 ppm is enough to inhibit the carcinogenesis in these tissues. In addition, we have found the protective effect of 2000 ppm of protocatechuic acid in diet against progression of tongue carcinogenesis.²³ Therefore, it is possible that less than 2000 ppm of this compound might inhibit all phases (initiation, promotion, and progression) of tongue carcinogenesis.

There are also reports showing negative chemopreventive activity of protocatechuic acid. In the experimental lung tumorigenesis of

Table 1 Evaluation of the activities of protocatechuic acid in preventing different chemical carcinogenesis in rodents

Species/strain/ gender of animals	Carcinogen/ promoter	Protocatechuic acid		Target tissue/ cancer	Response	Authors/yr (ref. no.)
		Dose/rout	Experimental protocol			
F344 rats/males	4-NQO	500, 1000, 2000/in diet	4-NQO + PCA and 4-NQO → PCA	Oral cavity (tongue)/SCC	Inhibition	Tanaka T et al/1994 ²²
F344 rats/males	4-NQO	2000/in diet	4-NQO → 4-NQO + PCA	Oral cavity (tongue)/SCC	Inhibition	Suzuki R et al/2003 ²³
Syrian golden hamsters/males	DMBA	200 ppm/in diet	DMBA → PCA	Buccal pouch/ SCC	Inhibition	Ohnishi M et al/1997 ²⁴
F344 rats/males	MNNG	1500 ppm/in diet	MNNG → PCA	Forestomach/SCC	No effects	Hirose M et al/1992 ³⁰
F344 rats/males	AOM	1000, 2000 ppm/in diet	AOM + PCA → PCA	Colon/ADC	Inhibition	Kawamori T et al/1994 ³³
F344 rats/males	AOM	250, 500, 1000 ppm/in diet	AOM + PCA and AOM → PCA	Colon/ADC	Inhibition	Tanaka T et al/1993 ²⁵
Syrian golden hamsters/females	BOP	500, 1000 ppm/in diet	BOP → PCA	Pancreas/ADC	Inhibition	Nakamura H et al/2000 ²⁶
F344 rats/males	DEN	500, 1000 ppm/p.o.	DEN + PCA and DEN → PCA	Liver/AD	Inhibition	Tanaka T et al/1993 ²⁷
A/J mice/females	NNK	1000 ppm/in diet	NNK + PCA and NNK → PCA	Lung/AD	No effects	Mori H et al/1999 ²⁹
F344 rats/males	BBN	500; 1000; 2000 ppm/in diet	BBN + PCA and BBN → PCA	Urinary bladder/TCC	Inhibition	Hirose Y et al/1995 ²⁸
CD-1 mice/females	B[a]P/TPA	5, 10, 20 μM/locally on the skin 5 min before TPA	DMBA → TPA + PCA	Skin/PAP	Inhibition	Tseng TH et al/1998 ¹⁶
ICR mice/females	DMBA /TPA	16, 160, 1600 nM/topically to the skin 0; 40 min or 3 hr before TPA	DMBA → TPA + PCA	Skin/PAP	Inhibition (16nM); no effects (160nM and 1600nM) Enhancement of skin papilloma by 1600nM PCA	Nakamura Y et al/2000 ³²
F344 rats	PhIP	2000 ppm /in diet	PhIP + PCA → PCA	Breast/ADC	No effects	Mori H et al/1999 ³¹

AD = adenoma; ADC = adenocarcinoma; AOM = azoxymethane; B[a]P, benzo[*a*]pyrene; BBN = *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine; BOP = *N*-nitrosobis(2-oxopropyl) amine; DEN = *N*-diethylnitrosamine; DMBA = 7,12 dimethylbenz[*a*]anthracene; MDMG = *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; NNK = 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone; 4-NQO = 4-nitroquinoline oxide; PAP = squamous cell papilloma; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; SCC = squamous cell cancer; TCC = transitional cell carcinoma; TPA = 12-*O*-tetradecanoylphorbol 13-acetate.

mice, induced by 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone, protocatechuic acid in diet at a dose of 1000 ppm during the initiation or promotion phase did not exert any protective effects on the development of lung tumors.²⁹ Protocatechuic acid at a dose of 2000 ppm was also ineffective in the prevention of cancers in the forestomach³⁰ and mammary gland³¹ of rats, induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*] pyridine, respectively.

Protocatechuic acid influences the development of squamous cell papillomas in skin cancers in the classic two-stage carcinogenesis model using 7,12-dimethylbenz[*a*]anthracene as an initiator and 12-*O*-tetradecanoylphorbol 13-acetate (TPA) as a promoter. Topical application of protocatechuic acid at small doses (<20nM) for 5 minutes before painting of TPA inhibited the incidence and number of papillomas in mice.¹⁶ In another experiment carried out in the same model, the effect of protocatechuic acid depended on the dose used and the timing between administration of the acid and TPA application. Protocatechuic acid at a dose of 16nM, which was applied 40 minutes before TPA treatment, caused a reduction in the number of tumors, whereas a dose of 1600nM increased the number of papillomas. Application of protocatechuic acid at a high dose (20,000nM) 5 minutes before TPA decreased the number of tumors, whereas administration 3 hours before TPA significantly increased the number of skin tumors in mice.³² Therefore, much attention should be paid to the administration dose or time of protocatechuic acid in human chemoprevention studies. These data indicate an organotropic activity of protocatechuic acid and suggest that possible cancer chemopreventive compounds need to be carefully examined for effectiveness in multiple organs by different models.

3. Mechanisms of Chemopreventive Action of Protocatechuic Acid

3.1. Effects of protocatechuic acid on the redox balance in cells

Chemopreventive action of protocatechuic acid is largely because of its antioxidant properties. Reactive oxygen species (ROS) and reactive nitrogen species, occurring as a result of exposure to environmental chemicals and radiation in the course of physiological or metabolic processes, may damage or modify macromolecules, that is, nucleic acids, structural proteins and enzymes, and membrane lipids. This may lead to mutation and disruption in the signaling pathways in the cell and, consequently, to the development of cancer. It has been shown that ROS and reactive nitrogen species can affect every stage of carcinogenesis, from initiation phase to the phase of promotion/progression.³⁵

The antioxidant activity of protocatechuic acid is well documented. Studies using *in vitro* cellular system and ROS generation have shown that protocatechuic acid inhibits both the formation of free radicals, including the highly reactive hydroxyl radical, and the scavenging of free radicals.^{37,38} Inhibition of the formation of free radicals is associated with the ability of protocatechuic acid to form complexes with transition metal ions, Cu (II) and Fe (II), or lowering the activity of enzymes catalyzing reactions in the course of which such radicals are formed, such as xanthine oxidase.^{39,40} The neutralization of free radicals is the result of their reaction with hydroxyl groups of protocatechuic acid. *In vitro* models showed that protocatechuic acid prevents oxidative DNA damage and lipid peroxidation.^{41,42}

The antioxidant activity of protocatechuic acid was also observed *in vitro* on cell cultures under conditions of oxidative stress.¹³ Protocatechuic acid was able to completely prevent the low-density lipoprotein (LDL) oxidation mediated by inhibited rat macrophage-like cells J774A.1.¹⁴ Protocatechuic acid inhibits the concentration of

reduced glutathione content and restores glutathione reductase and glutathione peroxidase activities. Protocatechuic acid also restores the mRNA levels of γ -glutamylcysteine synthetase, glutathione reductase, and glutathione peroxidase to control levels.¹⁴

Protocatechuic acid at concentrations 0.02–0.1 mg/mL and 50–100 mg/kg prevented the undesirable consequences of oxidative stress in the primary culture of rat hepatocytes and in liver of rats exposed to *tert*-butyl hydroperoxide (*t*-BHP).^{43,44} The findings demonstrated that protocatechuic acid reduced the cytotoxicity of *t*-BHP and raised the level of glutathione (GSH). Furthermore, protocatechuic acid inhibited lipid peroxidation, DNA repair processes, and oxidative damage induced by nucleic acid and prevented mitochondrial membrane depolarization. In addition, protocatechuic acid caused a reduction in phosphorylation of tyrosine residues of proteins of hepatocytes, suggesting an impact on signal transduction mechanisms in the cell.⁴³ It is known that one of the ROS and lipid peroxidation products—4-hydroxy-2-nonenal—can regulate the phosphorylation of signaling proteins by stimulating the tyrosine kinase activity.⁴⁵

Protocatechuic acid affects the oxidative stress in the skin of CD-1 mice that received local application of another inducer, TPA.¹⁶ Protocatechuic acid at doses of 5–20mM reduced the inflammation caused by the administration of TPA, inhibited the production of hydrogen peroxide (H₂O₂), and decreased the activity of myeloperoxidase in the skin. Myeloperoxidase present in neutrophils is responsible for the oxidation of certain carcinogens with H₂O₂, which plays an important role in their biotransformation.

However, protocatechuic acid, like many other well-known antioxidants, such as ascorbic acid and α -tocopherol, may exhibit pro-oxidant action under certain conditions.⁴⁶ Protocatechuic acid works as an antioxidant at low concentrations, whereas at high concentrations, it works as a pro-oxidant.^{32,47–49} Protocatechuic acid at high concentrations (>10mM) was demonstrated to induce oxidative stress in immortalized human gingival S-G epithelial cells and salivary gland cancer cells HSG1, as evidenced by the intensity of lipid peroxidation in the presence of Fe (II) and lower levels of GSH. Protocatechuic acid at a concentration of 2.5nM potentiated the toxicity of *t*-BHP.⁴⁷ Similar observations were reported in some *in vivo* studies. Intraperitoneal administration of protocatechuic acid at a toxic dose (500 mg/kg) reduced the concentration of GSH in the liver and the kidney of ICR mice.⁴⁹ Dose-dependent effect of protocatechuic acid was confirmed by alterations in the skin of mice after exposure to TPA.^{32,48} Several independent *in vivo* experiments showed that when given protocatechuic acid at doses of 1600 and 20,000nM for 0.5 or 3 hours before application of TPA, the oxidative stress and inflammation were potentiated in the skin. Increased levels of lipid peroxidation, H₂O₂ generation, and myeloperoxidase activity, and decreased levels of GSH were also observed. Use of 16nM protocatechuic acid was protective against oxidative stress and papilloma formation in the skin. Results of these *in vivo* studies and *in vitro* works using human promyelocytic leukemia cells HL-60 suggested that the effects of higher doses of protocatechuic acid may be associated with the oxidation of the chemical by tyrosinase, which leads to a more reactive derivative, probably quinone that can react with glutathione and proteins. The consequence is the loss of GSH and disruption of detoxification systems, and modifications—sometimes necessary to proteins—which can induce a local immune response increasing the effects of oxidative stress.

3.2. Effect of protocatechuic acid on the metabolism of carcinogens

The chemopreventive action of protocatechuic acid is also linked to its effects on the metabolism of carcinogens. The process involves two groups of enzymes, Phase I and Phase II. Phase I biotransformation enzymes are mainly from the superfamily of cytochrome

P-450 and catalyze hydroxylation reactions. In the course of this transformation, metabolic activation can occur, and the resulting metabolites may react with the DNA and form adducts. In contrast, Phase II enzymes that detoxify carcinogens catalyze the conjugation of glucuronic acid, sulfuric acid, or glutathione. This increases the solubility of these compounds in water and facilitates excretion. However, these reactions can also lead to the activation of some carcinogens. This group includes GST, uridine 5'-diphosphate (UDP)-glucuronosyltransferase, and reduce nicotinamide adenine dinucleotide phosphate (NAD(P)H):quinone (NQO1).⁵⁰ It has been shown that protocatechuic acid has an effect on enzymes involved in both Phase I and II biotransformation of carcinogens.^{51–54}

Protocatechuic acid inhibited the catalytic activity of certain cytochrome p-450, especially CYP1A2 and, to a lesser extent, CYP1A1 and CYP2B, induced by sodium phenobarbital or 5,6-benzophenone in microsomes from mouse hepatocytes of *in vitro* study.⁵¹ *In vivo* studies have shown that a single administration of protocatechuic acid to rats have stronger effects on the activities of Phase I and II enzymes than subchronic administration of protocatechuic acid to rats at a dose of 250- or 500-mg/kg body weight. Exposure of protocatechuic acid reduced the activities of CYP1A1, CYP1A2, and CYP2B in the liver but only CYP2B in microsomes obtained from kidney homogenates. Giving protocatechuic acid 1 hour before the application of *o*-toluidine, which is an aromatic amine metabolizable by CYP1A1, caused increases in the activities of CYP1A1 and CYP1A2 in the liver and an increase in CYP1A1 activity only in the kidney. Protocatechuic acid also increased the activity of GST, which was reduced after the administration of *o*-toluidine.⁵⁴ However, protocatechuic acid administered at a dose of 50 mg/kg every 3 days for 2 weeks to rats that were exposed to 3-methylcholanthrene at the 12th day of protocatechuic acid treatment resulted in decreases in activities and expression of CYP1A1, CYP1A2, and CYP2E1, and an increase in GST and NQO1 activities in the livers of animals.⁵⁵ Protocatechuic acid also reduced the constitutive activity induced by 3-methylcholanthrene and isoenzyme CYP2E1 in the kidneys of rats. Administration of protocatechuic acid had no effect on cytochrome P-450 activity, whereas it decreased the activity of detoxification enzymes studied, that is, GST, UDP-glucuronosyltransferase, and NQO1, in the livers of animals.

These results indicate that protocatechuic acid not only affects the activities of enzymes involved in the metabolism of carcinogens, but also neutralizes reactive intermediate metabolites, thereby preventing their binding to DNA. Blocking the DNA-binding site with carcinogens by protocatechuic acid is likely to prevent DNA mutations and tumor initiation.⁵² Different effects of protocatechuic acid on the activity of enzymes involved in the biotransformation of the studied organs may be partly explained by differences in the efficacy of phenol in the prevention of chemically induced carcinogenesis in experimental animals.

3.3. Other mechanisms of anticarcinogenesis action of protocatechuic acid

Protocatechuic acid possesses antiproliferative action on several human cell lines, including immortalized breast cells HBL 100, breast cancer cells T47D, gastric adenocarcinoma cells MKN45, lung cancer cells PC14, and promyelocytic leukemia cells HL-60.^{8,56–58} Zheng et al.⁵⁹ reported that protocatechuic acid exerts tumor-preventive action through apoptosis- and cell proliferation-independent mechanisms in human colon cancer cell lines. Inhibition of the proliferation of nonlesional epithelial cells, including those of oral cavity,^{22,24} colon,²⁵ liver,²⁷ and bladder,²⁸ was also observed in chemical carcinogenesis models.

Antiproliferative action of protocatechuic acid can be derived from its antioxidant properties. Some ROS, such as H₂O₂, act as a second messenger in cell signal. They can activate transcription factors, such as nuclear factor kappa B (NF- κ B)^{60–63} or activator protein-1, and thus, affect the expression of genes involved in cell cycle regulation and apoptosis.³⁵ Interestingly, protocatechuic acid suppresses the expression of inducible nitric oxide synthase (iNOS)⁶¹, cyclooxygenase-2,⁶¹ and tumor necrosis factor (TNF)- α ,⁶³ which are involved in carcinogenesis and/or inflammation.

Polyamines, including putrescine, spermine, and spermidine, play an important role in the growth and differentiation of cells by interfering with the mechanisms of signal transduction.⁶⁴ Polyamines induce a cascade of kinases, such as tyrosine kinase and mitogen-activated protein kinase, and stimulate the transcription of some oncogenes, such as *c-myc*, *c-jun*, and *c-fos*. Protein products of these oncogenes function as transcription factors and may stimulate the proliferation of cells. The main enzyme in polyamine biosynthesis is ornithine decarboxylase, which catalyzes the conversion of ornithine to putrescine. Increased enzyme activity of ornithine decarboxylase was observed in many cancers and in precancerous changes as well. Protocatechuic acid reduces the enzymatic activity of ornithine decarboxylase in target organs and levels of polyamines in serum in some chemically induced carcinogenesis models in rodents.^{22,23,25,27,33}

A recent study has shown that protocatechuic acid has a direct effect on the process of DNA replication. Stagos et al.¹⁵ demonstrated that protocatechuic acid is a potent inhibitor of topoisomerase I, the enzyme responsible for the catalytic reaction of cutting and joining DNA polynucleotide chains.

Protocatechuic acid also affects apoptosis to eliminate damaged and neoplastic cells.^{65,66} In human promyelocytic leukemia cell line HL-60, protocatechuic acid increased the proportion of cells in the G1 phase of the cycle and induced apoptosis; treatment with protocatechuic acid increased the level of hypophosphorylated RB protein and the expression of proapoptotic protein, BAX, whereas the treatment caused a decline in hyperphosphorylated RB and a decrease in the expression of antiapoptotic protein Bcl-2.⁵⁸ These data suggest that protocatechuic acid is an apoptosis inducer in human leukemia cells, and that RB phosphorylation and Bcl-2 protein may play a crucial role in the early stage. It should be pointed out that RB protein plays an important role in the regulation of cell cycle and apoptosis. At the end of G1 phase, after phosphorylation by protein complexes of cyclin D-dependent kinase 4/6 and cyclin E-dependent kinase 2, transcription factors are released from the E2F family, which after joining the heterodimeric DNA binding protein partners called DP are associated with promoter areas of target genes, stimulating the transcription of cells and the transition into a new phase of the cycle. However, poorly phosphorylated RB protein binds E2F factors, which prevents the gene transcription and stops the cell cycle in G1 phase.⁶⁷ Regardless of stopping the cycle, RB protein directly inhibits the transmission of signals in cell death.³⁷ Reduction of the levels of both forms of proteins 9 hours after administration of protocatechuic acid may be associated with the inhibition of polyamine biosynthesis by caspases and degradation, which in turn, triggers apoptosis.⁵⁸ Recent studies indicate, however, that some of the reduced RB protein molecule can inhibit apoptosis.⁶⁸

There have been reports showing that protocatechuic acid prevents apoptosis initiation by affecting death receptors. In human umbilical vein endothelial cell line and T-cell lymphoma cell line Jurkat, protocatechuic acid inhibited apoptosis induced by TNF- α .⁶³ Protocatechuic acid also increased the activity of the transcription factor NF- κ B as a consequence of worsening degradation of the inhibitory protein I κ B α , which creates cytosolic complex with NF- κ B and prevents its translocation to the nucleus. After

phosphorylation of I κ B α by a specific kinase released NF- κ B moves to the nucleus and combines with an element in the areas of I κ B promoter of target genes, including those antiapoptosis.

Protocatechuic acid also inhibited hepatocyte apoptosis induced by TNF- α in *in vivo* studies of rodents.^{38,69,70} Sepsis induced by bacterial lipopolysaccharide administration causes the release of free radicals and various cytokines—mediators of inflammation—which leads to a substantial damage in a variety of tissues. Exponential liver damage in the course of endotoxin shock is hepatocyte apoptosis, which triggers the binding of proinflammatory cytokines TNF- α and TNF receptor 1 (p55). Protocatechuic acid isopropyl ester decreased the level of TNF- α and increased the level of anti-inflammation of interleukin-10.³⁸ This process was accompanied by increased expression of iNOS in hepatocytes, increased production of iNOS, and increased levels of nitrogen compounds in the blood. Protocatechuic acid and its derivatives, such as isopropyl ester of protocatechuic acid, are demonstrated to counter these phenomena.^{37,69}

It is important to evaluate the toxicity of chemopreventives for use in humans with a high risk of cancer.^{1,2} In our preliminary study for determining the maximum tolerated dose of protocatechuic acid, the value was more than 10 g protocatechuic acid/kg basal diet (10,000 ppm). Rats fed protocatechuic acid-containing diet at this dose for 6 weeks did not show clinical signs or histopathological changes for toxicity, weight gain retardation, or abnormalities of chemical profiles.²² The lowest dose of protocatechuic acid that effectively acted as a cancer chemopreventive agent was 500 ppm. Phenolic compounds are ubiquitous in edible vegetables, fruits, and nuts. It is estimated that an average of 1–2 g/d of phenolic compounds is consumed by humans.⁷¹ Protocatechuic acid is a widely distributed phenolic acid. Several kinds of fruits, vegetables, and plants, such as citrus fruit and fennel, contain a small amount of protocatechuic acid. The lowest level of protocatechuic acid found to be effective in inhibiting tumorigenesis is almost four times greater than that consumed by humans, assuming that 10 g lettuce and/or strawberries, which contain 10–40 mg protocatechuic acid/100 g, are consumed daily. Further research is needed to better understand the underlying mechanisms of chemopreventive action and the pharmacokinetic absorption, distribution, metabolism, and excretion (ADME) data of protocatechuic acid.

4. Conclusion

Protocatechuic acid is one of the biologically active substances isolated from a number of popular medicinal plants growing in different parts of the world.^{9,16,18,19,41,57} Research conducted over the past several years indicates that it may be used in conventional medicine to prevent cardiovascular diseases and cancer.^{13–15,21} The mechanism of the preventive action of protocatechuic acid is based on its antioxidant properties, that is, inhibition of the generation of free radicals, and their ability to scavenge and increase the catalytic activity of endogenous enzymes involved in the neutralization of free radicals. It is important that the impact of protocatechuic acid on the activity of enzymes involved in Phase I and II biotransformation of carcinogens and, possibly, direct blocking of specific binding sites of carcinogens with DNA molecule. Other aspects regarding the impact on the activity of cyclooxygenase-2, iNOS, inflammatory cytokines, and the proteins regulating cell cycle process are poorly understood. Our inflammation-associated colon carcinogenesis model (Tanaka model)^{72,73} is useful in investigating the effects of protocatechuic acid and other compounds, which are candidate chemopreventive agents, on the expression of these molecules and proteins during carcinogenesis.^{74–76} The safety of protocatechuic acid in humans should be considered based on the

reports that the chemical increased oxidative stress and the number of certain types of tumors in experimental animals.^{32,47–49} However, both effects have been observed using only very high doses of the chemical. Daily consumption of protocatechuic acid was not clearly defined, but we should take into account the fact that its content in food rarely reaches 2–10 mg/g; hence, it is probably much less than 1 mg/kg. The dietary dose of protocatechuic acid is, therefore, at least 100–500 times smaller than those that caused side effects, which is described in several reports.^{9,11,49} However, protocatechuic acid may be one of the active metabolites of plant phenolic compounds with more complex structural construction, such as anthocyanins, and therefore, its concentration in the human body may be higher than that in the acid content in the products consumed.⁴⁴ When considering the development of chemoprevention strategy with protocatechuic acid in humans, we should take into account its content both in the daily diet and in other natural or synthetic antioxidant compounds consumed, which may increase the effects of protocatechuic acid under certain conditions.

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