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ScienceDirect
Mutation Research 638 (2008) 175–183
Fundamental and Molecular Mechanisms of Mutagenesis
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Gallic acid, a metabolite of the antioxidant propyl gallate, inhibits gap junctional intercellular communication via phosphorylation of connexin 43 and extracellular-signal-regulated kinase1/2 in rat liver epithelial cells

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Received 3 July 2007; received in revised form 7 October 2007; accepted 8 October 2007
Available online 22 October 2007

Abstract

Propyl gallate and its metabolite, gallic acid, are widely used as antioxidants in the food industry, but they have been shown to exhibit liver toxicity and enhance carcinogenesis. In the present study, we investigated the possible undesirable effects of propyl gallate and gallic acid on gap junctional intercellular communication (GJIC), inhibition of which is closely linked to carcinogenesis. Gallic acid and propyl gallate exhibited dose-dependent free-radical-scavenging activities as determined by 1,1-diphenyl-2-picrylhydrazyl- or 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)-radical-scavenging assays, and the free-radical-scavenging activity of gallic acid was stronger than that of propyl gallate. However, using WB-F344 rat liver epithelial cells, gallic acid inhibited GJIC in a dose-dependant manner, while propyl gallate had no significant effect compared with untreated controls. The gallic-acid-induced inhibition of GJIC was reversible, with a recovery of nearly 65% after 120 min. Gallic acid induced the phosphorylation of connexin 43 (Cx43) and phosphorylation of extracellular-signal-regulated kinase1/2 (ERK1/2). The gallic-acid-induced inhibition of GJIC was attenuated by treatment with mitogen-activated protein kinase kinase inhibitors (U0126 and PD098059). U0126 blocked the gallic-acid-induced phosphorylation of Cx43 and ERK1/2, indicating that the gallic-acid-induced inhibition of GJIC is mediated by phosphorylation of Cx43 via activation of ERK1/2. In addition, gallic-acid-induced inhibition of GJIC was protected by ascorbic acid and quercetin, which might represent a simple example of the different effects of natural antioxidants in carcinogenesis.

Keywords: Connexin 43; Extracellular-signal-regulated kinase; Gap junctional intercellular communication; Metabolite; Propyl gallate

1. Introduction

The positive effects of dietary antioxidative phenolics have been widely investigated, but their negative effects remain unclear. Several studies have indicated contradictory effects of dietary antioxidative phenolics [1,2]. Thus, the effect of dietary phenolic phytochem-

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doi:10.1016/j.mrfmmm.2007.10.005

icals on carcinogenesis may vary with the structure and dose of the individual compounds. Propyl gallate (Fig. 1) is widely used as an antioxidant in the food industry, and several investigations have shown its potential chemopreventive effects in animal experiments [3–5]. Thus, propyl gallate has been recognized as an important synthetic antioxidant. However, propyl gallate enhances the mutagenic activities of the carcinogens *N*-hydroxy-2-acetylaminofluorene and 4-nitroquinoline 1-oxide, as determined by a salmonella/microsome mutagenesis assay [6]. Propyl gallate also induced sister-chromatid exchanges and chromosomal aberrations in CHO-K1 cells [7], and coadministration of propyl gallate with sodium nitrite promoted forestomach carcinogenesis after its initiation with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine in rats [8]. The National Toxicology Program reported that propyl gallate induces preputial gland tumors, islet-cell tumors of the pancreas, pheochromocytomas of the adrenal glands in male rats, and malignant lymphoma in male mice [9]. Propyl gallate is not easily excreted, and hence tends to accumulate in the body [10]. Together these reports suggest that the accumulation of propyl gallate contributes to carcinogenesis, but the underlying mechanism remains unclear.

Gallic acid (Fig. 1), a metabolite of propyl gallate, exerts strong antioxidant activity [11] and exerts antiproliferative effects on cancer cells by generating hydrogen peroxide [12]. However, a recent study demonstrated that gallic acid produced metal-mediated oxidative damage in cellular and isolated DNA [13]. It was found that intraperitoneal injection of gallic acid at 500 mg/kg into CD-1 mice increased plasma alanine aminotransferase levels by four fold after 24 h [14], indicating that significant hepatic damage is induced by gallic acid *in vivo*. Recently we reported that gallic acid exhibited cytotoxicity in normal rat liver epithelial cells [15].

Gap junctional intercellular communication (GJIC) is a mechanism of direct cell-to-cell signaling that is mediated by gap junctions, which consist of transmembrane proteins called connexins. GJIC plays a critical role in tissue development and differentiation, and is important to the maintenance of tissue homeostasis through the exchange of ions, signaling molecules, nucleotides, and other small molecules (less than 1 kDa) between adjacent cells [16]. Dysfunction of these gap junctions might play a role in the actions of various toxic chemicals that have cell type, tissue, or organ specificity [17]. Thus, GJIC is a useful model for examining the toxicities of chemicals *in vitro* and *in vivo*. It is well known that GJIC is dysfunctional in most cancer cells [18], and that inhibition

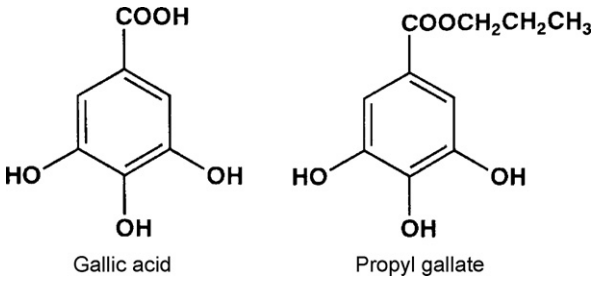

Fig. 1. Chemical structures of gallic acid and propyl gallate.

of GJIC by tumor promoters is strongly involved in carcinogenic processes, particularly in the tumor-promotion stage [17,19]. Diminished GJIC plays an important role in both the progression and promotion of carcinogenesis. The present study investigated the possible inhibitory effects of gallic acid on GJIC and the underlying molecular mechanisms.

2. Materials and methods

2.1. Chemicals

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin were purchased from GIBCO BRL (Carlsbad, CA, USA). Ammonium phosphate monobasic ($NH_4H_2PO_4$), dimethyl sulfoxide (DMSO), gallic acid, propyl gallate, ascorbic acid, quercetin, hydrogen peroxide, U0126, PD098059, lucifer yellow, ammonium ferrous sulfate, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), sodium dodecyl sulfate (SDS), acrylamide, and Tris–HCl were obtained from Sigma Chemical (St. Louis, MO, USA). Triton X-100 was obtained from AMRESCO (Solon, OH, USA). Antibodies to extracellular-signal-regulated kinase1/2 (ERK1/2) and phosphorylated ERK1/2 (p-ERK1/2) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and anti-connexin 43 (Cx43) antibody was obtained from Zymed Laboratories (San Francisco, CA, USA). A protein assay kit was from Bio-Rad Laboratories (Hercules, CA, USA) and all other chemicals used were of analytical or HPLC grade from Sigma Chemical or Fisher Scientific (Springfield, NJ, USA).

2.2. DPPH-radical-scavenging-activity assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH)-radical-scavenging activities of gallic acid and propyl gallate were measured using the method described by Brand-Williams et al. with minor modifications [20,21]. DPPH radical was dissolved in 80% aqueous methanol. One hundred microliters of the solution containing gallic acid or propyl gallate at various concentrations was added to 2.9 ml of the DPPH-radical solution. The mixture was then shaken vigorously and allowed to stand at 23 °C in the dark for 30 min, at which time the