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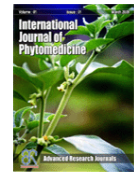

Short Communication

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A supposed mechanism of synergistic action of catechol-containing natural polyphenols

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Abstract

Over the past decades, accumulated evidences have been published about different synergistic biological activities between natural dietary polyphenols. Although these effects could be physiologically important in chemoprevention, cardioprotection and neuroprotection, but probably also in treatment of serious chronic diseases, such as cancer, the exact mechanisms behind this potentiation have still remained largely unknown. In this article, supposition about the involvement of phase II metabolic enzyme, catechol-O-methyltransferase (COMT), in the synergistic action of catechol-containing polyphenols is proposed. Serving as substrates, these compounds can also behave as COMT inhibitors suppressing the O-methylation of the other catechol-containing component in the combined mixture. At that, negative feedback by the increased amount of S-adenosyl-L-homocysteine generated from the methyl-group donor S-adenosyl-L-methionine during the enzymatic conversion can play an important role. Presuming that O-methylated conjugates are in general biologically less active than their unmetabolised counterparts, cotreatment of cells with combination of two catecholic natural agents can lead to a superior effect as compared to the administration of either compound alone. This mechanism can provide an explanation to the beneficial synergistic effects described for green tea extracts in chemoprevention or red wine consumption in protection of cardiovascular system in comparison with their single components tested separately. However, as currently only little is known about the possible biological activities of O-methylated conjugates of dietary polyphenolic phytochemicals, their nature and effects definitely need to be further studied. These results could prove (or disprove) the hypothesis raised in this article but also contribute to the development of physiologically or even clinically useful mixtures of polyphenols with catechol structure in the future.

Keywords: Catecholic phytochemicals, Catechol-O-methyltransferase; Chemoprevention; Cytotoxicity; Flavonoids; Synergistic bioactivities

Introduction

Natural dietary polyphenolic agents, including flavonoids and phenolic acids, have been consumed with safety for centuries and numerous preclinical investigations suggest that many of them exert chemopreventive, cardioprotective and neuroprotective properties [1-5]. Various experimental and epidemiological evidences demonstrate that flavonoids exhibit antioxidant, anti proliferative, proapoptotic, anti inflammatory, antiangiogenic, and antimetastatic effects to inhibit development and growth of various tumors [2,6-8]. Flavonoids can be found abundantly in plant-based food items, such as fruits, vegetables, nuts, seeds and medicinal herbs, and they are usually consumed in different combinations in variable amounts [4]. However, the mechanisms of interactions between such dietary compounds are far from being completely understood [9,10] and definitely need further unraveling for more efficient preventive and therapeutic applications in the future.

Combining polyphenolic dietary agents in mixtures can increase or decrease their biological activities, revealing as additive, synergistic or antagonistic effects [11]. Indeed, in our recent experimental article, we demonstrated that two flavonols, fisetin (3,7,3´,4´-tetrahydroxyflavone) and quercetin (3,3´,4´,5,7-pentahydroxyflavone), potentiated the cytotoxic activity of luteolin (3´,4´,5,7-tetrahydroxyflavone) in human chronic lymphocytic leukemia cell lines HG-3 and EHEB [12]. All these three compounds contain catechol moiety in their molecules (Fig. 1). On the contrary, hesperetin (3´,5,7-trihydroxy-4´-methoxyflavanone), chrysin (5,7-dihydroxyflavone) and baicalein (5,6,7-trihydroxyflavone) without such a catechol structure exerted no augmentation of luteolin cytotoxic action in these cells [12]. Several other examples about synergistic action of catechol-containing

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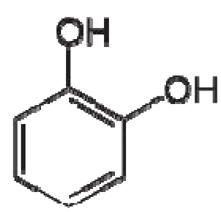
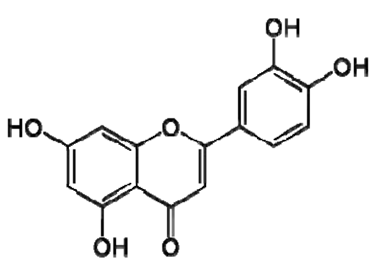
polyphenols can be found in literature. Amin et al. described the synergistic apoptotic effects between luteolin and green tea catechin epigallocatechin gallate (EGCG) in various head and neck and lung cancer cell lines [1]. Moreover, this combination considerably suppressed the tumor growth also in mouse xenograft models [1]. Suganuma et al. reported synergistic induction of apoptosis and growth inhibition in cotreatment of human lung cancer cells PC-9 with EGCG and another green tea catechin, epicatechin (EC). This augmentation was explained by the enhanced cellular incorporation of tea flavanols [9]. The same combination of catechins (EGCG plus EC) was shown to display synergistic action on growth inhibition and apoptosis also in human colon cancer cell line HT29 [10]. Xu et al. reported synergistic action on apoptosis and proliferation of human prostate cancer cell line PC-3 when combining myricetin (3,3´,4´,5,5´,7-hexahydroxyflavone) and myricitrin (3-O-rhamnoside of myricetin), both these flavonols contain catechol structure element [2]. Furthermore, the combination of EGCG with quercetin was demonstrated to synergistically inhibit the self-renewal properties of human prostate cancer stem cells by inducing apoptosis, suppressing viability and limiting migration and invasion of cancer stem cells, by that contributing to eradication of tumor and prevention of its recurrence [7]. In addition to targeting malignant cells, mixtures of catechol-containing flavonoids have been demonstrated to synergistically affect also surrounding tumor microenvironment resulting in prevention of neoplastic progression. Indeed, combinations of EGCG and luteolin inhibited prostate cancer-related myofibroblast phenotype and activation in increased efficacy as compared to either compound alone, thereby suppressing extracellular matrix contraction and invasion of malignant cells [8].

Combinations of catechol-containing natural polyphenols have been shown to regulate also other (patho)physiological processes. Pignatelli et al. described a synergistic inhibition in adhesion of human platelets to collagen and collagen-induced platelet aggregation when combining catechin with quercetin [3]. Redondo et al. showed that coincubation of mesenteric smooth muscle cells obtained from spontaneously hypertensive rats with these two flavonoids led to a significant suppression of angiotensin II-induced production of reactive oxygen species, cellular proliferation and migration at doses where quercetin and catechin alone were ineffective, providing thus vascular protection [4]. Park et al. described a synergistic reduction of various inflammatory mediators, i.e. production of nitric oxide and prostaglandin E2, levels of tumor necrosis factor- and interleukin-1β, expression and enzymatic activity of inducible nitric oxide synthase and cyclooxygenase-2, by cotreatment of lipopolysaccharide-stimulated murine RAW 264.7 macrophages with luteolin and chicoric acid. This combined intervention with nutraceutical agents can be a useful tool for inflammatory diseases [11,13].

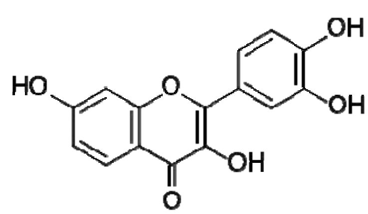
Chemical structures of the above mentioned natural polyphenols are presented in the Figure 1. It can be seen that all these phytochemicals contain a catechol structure element.



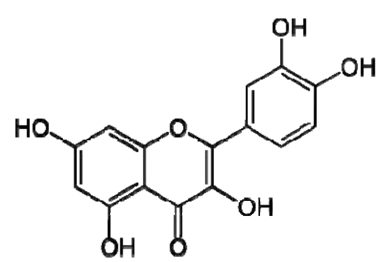
Catechol moiety



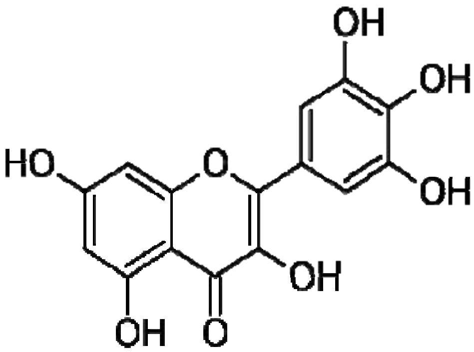
Luteolin



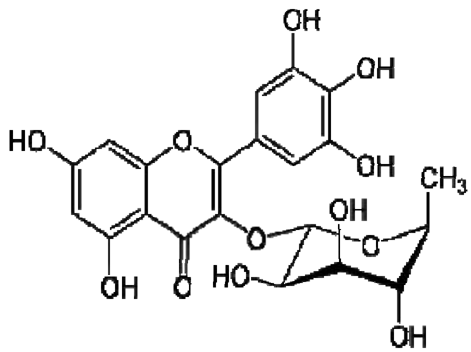
Fisetin



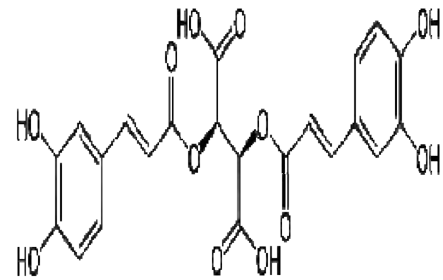
Quercetin



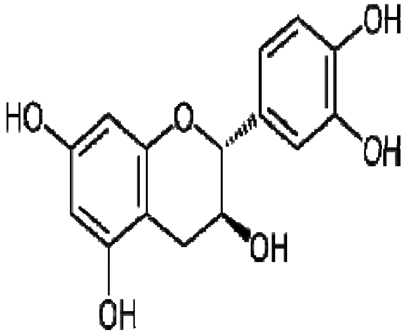
Myricetin



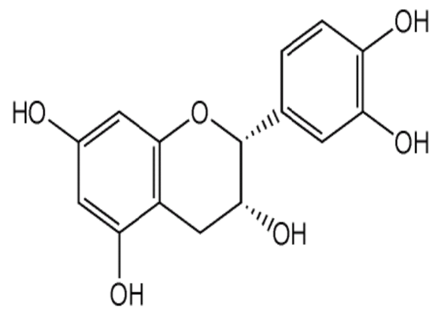
Myricitrin



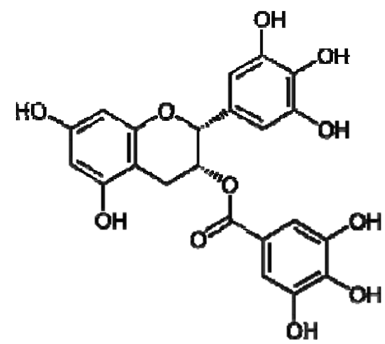
Chicoric acid



(+)-Catechin



(-)-Epicatechin



Epigallocatechin gallate

Figure 1. Structure of catechol group and examples of natural dietary polyphenols containing catechol structure

A supposed explanation of synergistic action

Despite the interesting synergistic effects between catechol-containing natural polyphenols, the mechanism of these interactions is remained largely unidentified. This let me to propose a hypothesis of involvement of catechol-O-methyltransferase (COMT)-catalyzed O-methylation reaction in these mutually enhanced activities. COMT is a phase II enzyme that catalyzes the addition of a methyl moiety to one of the hydroxyl groups in catechol structure leading to the formation of O-methylated derivative [14-17]. COMT activity is highest in the liver and kidneys, but this enzyme is widely distributed in practically all mammalian tissues [14]. The O-methylation reaction is dependent on the presence of S-adenosyl-L-methionine (SAM) as the methyl donor that is subsequently converted to S-adenosyl-L-homocysteine (SAH) [14]. SAH, in turn, is known as an inhibitor for COMT by representing a negative feedback mechanism [14,16,18-20]. Therefore, during the cotreatment of cells with two catechol-containing substances (marked as Polyphenol1 and Polyphenol2 in Figure. 2) the increased levels of SAH formed within the metabolic O-methylation of Polyphenol1 (or a decreased availability of SAM) can trigger an inhibition of COMT-catalyzed conversion of Polyphenol 2, according to the respective reaction kinetics. This schematic mechanism is depicted in Figure. 2. It is evident that the biological activities of polyphenols and their O-methylated derivatives can be remarkably different with conjugates being usually less effective, the position of the O-methylation (para- or meta-methylation) may also affect the cellular responses [21]. Thus, treating the cells with combinations of COMT substrates may change the ratio of original (active) catechol-containing phytochemicals and their (inactive) metabolic conjugates, leading to a potential synergism as compared to the administration of either dietary polyphenolic agent alone.

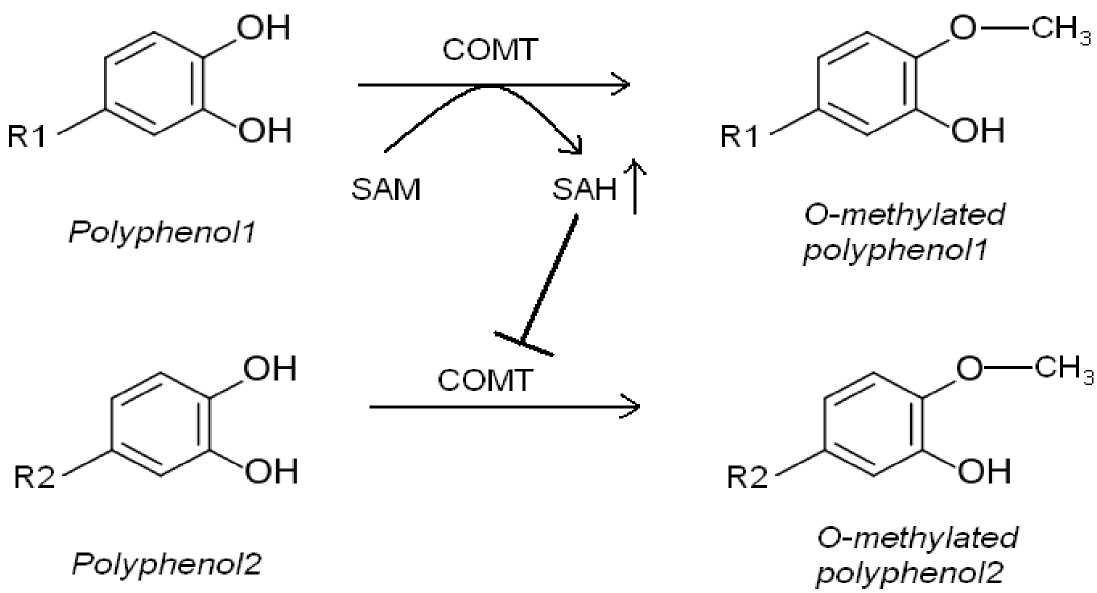


Figure 2. A hypothetic mechanism to explain the synergistic action between catechol-containing polyphenolic compounds (COMT, catechol-O-methyltransferase; SAH, S-adenosyl-L-homocysteine; SAM, S-adenosyl-L-methionine)

Supportive data and results

Several experimental data can be found in the literature to support this hypothesis. First, it has been shown that COMT converts the green tea flavanol EGCG mainly to 4´´-O-methyl-EGCG and to a lesser extent to 4´,4´´-di-O-methyl-EGCG; both of these metabolites possess considerably less anticancer properties than the unmethylated EGCG [22,23]. Wang et al. demonstrated that after intake of 6 cups of green tea daily during 3-5 weeks, about 50% of EGCG was present in the form of 4´´-O-methyl-EGCG in human prostate tissue derived at prostatectomy [24], whereas in cultured LNCaP human prostate cancer cells the capacity of 4´´-O-methyl-EGCG to suppress tumor cell growth and induce apoptosis was significantly lower compared to intact EGCG [24]. Thus, O-methylation can diminish the anticancer potential of green tea polyphenols and this is consistent with the evidences that suppression of the O-methylation of EGCG may increase its biological effects. Indeed, Landis-Piwowar et al. demonstrated in MDA-MB-231 human breast cancer cells that pharmacologically suppressed COMT activity by dinitrocatechol appeared to enhance the stability and anticancer bioactivities of EGCG [25]. Forester et al. recently showed that cotreating of H1299 human lung cancer cells and CL-13 murine lung cancer cells with EGCG and nitrocatechols tolcapone or entacapone, clinically approved COMT inhibitors in management of Parkinson`s disease, led to a synergistic inhibition of tumor cell viability. These COMT inhibitors suppressed the O-methylation of EGCG remaining higher levels of unmetabolised flavanol to reveal its anticancer activities as compared to the treatment with EGCG alone [22].

On the other hand, numerous studies have shown that catechol-containing dietary polyphenols may behave as potent COMT inhibitors by suppressing the O-methylation of a variety of catechol substrates. Zhu et al. demonstrated in their numerous studies that dietary phytochemicals like quercetin, fisetin, and tea polyphenols inhibited the O-methylation of endogenous metabolites of estradiol, i.e. catechol estrogens, and explained this process by a combination of several mechanisms, including the direct competitive inhibition of the enzyme by serving itself as a substrate and, as a major mechanism, the non-competitive inhibition resulting from elevated levels of SAH generated during the O-methylation of catecholic polyphenols; decreased availability of SAM was also shown to be involved [14,15,26-30]. SAH is known as a significant feedback inhibitor for COMT-mediated O-methylation of different catechol-containing compounds [15,16,27,30].

Furthermore, when human non-small cell lung adenocarcinoma A549, human renal cell adenocarcinoma 786-O and human liver hepatocellular carcinoma HepG2 cells were cotreated with EGCG and quercetin, the amount of 4``-O-methyl-EGCG was substantially diminished with comparison to administration of EGCG alone, showing that quercetin suppressed the COMT-catalyzed O-methylation of EGCG [31]. Similarly, the same combination of catechol-containing polyphenols increased the cellular doses of EGCG for almost ten-fold in human prostate cancer cell lines PC-3 and LNCaP [32]. These *in vitro* results were confirmed also in mouse study as the portion of unmetabolised EGCG was substantially increased in lung and kidney tissues when cotreated together with quercetin [31]. Moreover, quercetin was demonstrated to increase the growth inhibitory action of EGCG in A549, 786-O, HepG2, LNCaP and PC-3 cells, whereas the extent of this effect depended on the cellular expression and activity of COMT [31,32].

These data convincingly show that increase in the bioavailability of Polyphenol2 by suppressing the activity of COMT and thereby reducing the formation of its less active O-methylated metabolites by the other component in the mixture, i.e. Polyphenol1 (Figure. 2), may indeed ultimately lead to a synergistic biological activity.

Consequences of the hypothesis and discussion

The proposed mechanism can, at least in part, explain the different synergistic actions of catechol-containing dietary polyphenols described above. However, implications of this mechanism can be much more far-reaching and biologically significant.

It is well known that due to an extensive metabolic conversion, *in vivo* concentrations of polyphenols achieved after oral intake of plant-based food items are much lower than the doses shown to be effective in *in vitro* experiments which may also be associated with several side effects. Therefore, combination of natural dietary agents acting via a synergistic mechanism might decrease the concentrations of individual components revealing a potential biological importance at physiologically achievable doses [1-3,33].

Such potentiating action between catechol-containing dietary polyphenolic agents might be important in chemoprevention. Indeed, it has been shown that whole green tea or a well-defined mixture of different catechins, known as Polyphenon E, may display superior anticancer activities to its single components tested alone [9,10,33]. Moreover, combinations of natural polyphenolic agents have been shown to exert a synergistic action even on eradication of cancer stem cells or prevention of neoplastic progression via targeting the tumor microenvironment. In addition to chemoprevention, synergistic action between catechol-containing dietary polyphenols may be physiologically significant also in reduction of risk of cardiovascular diseases through moderate consumption of red wine [3]. The possible involvement of COMT-mediated O-methylation reaction in synergistic activities of complex mixtures remains to be determined. However, it is clear that combinatorial chemopreventive and cardioprotective strategies are gaining elevating popularity [1].

Although it is generally accepted that polyphenolic compounds undergo an extensive metabolic conjugation *in vivo* after their oral consumption, it is still relatively little known about the biological activities of different metabolites, including O-methylated conjugates. Therefore, to understand the synergistic mechanisms more detail, it is also crucial to determine the nature and potential biological effects of metabolites, especially compared to the parent compounds. Last but not least, it is clear that combinations of dietary phytochemicals which reveal beneficial synergistic actions in laboratorial experiments are certainly worth of further trials and probably also clinical developments.

Conflict of interest statement

None declare

Acknowledgements

None declare

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