Absorption, metabolism and protective role of fruits and vegetables polyphenols against gastric cancer

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Abstract. – Growing evidence links free radicals to the aging processes, degenerative diseases and cancer, underlying the important role played by some antioxidants, as polyphenols, present in fruits and vegetables, which seem able to counteract the toxic effects induced by oxidative stress. The gastrointestinal tract is continuously exposed to oxidant and antioxidant substances and, in particular in this district, the food rich in antioxidants could exert a protective effect against the risk of cancer. Polyphenols have a direct protective effect on the gastrointestinal tract, detoxifying the Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), preserving antioxidant proteins and complexing metals. Although polyphenols are a class of antioxidant largely represented in vegetables and fruits, we are still uncertain whether the beneficial effects of a diet rich in plant products, are mainly due to these compounds. Our knowledge does not allow to be sure about which antioxidants are capable of having therapeutic effects, through which mechanism, the exact therapeutic dose or how long they have to be taken to have a significant protective effect. In this review we take into account the most common antioxidants, usually found in the diet and the processes regulating their absorption, metabolism and excretion, in order to elucidate the mechanism that could be responsible for the protection against cancer.

Key Words:
   Oxidative stress, Gastric cancer, Polyphenols, Antioxidants metabolism, Polyphenols in fruit and vegetables.

Introduction

Free radicals are mainly constituted by unstable substances able to react with a large number of molecules, including those present in the cells, altering their structure and function[1]. Consequently, these interactions can lead to toxic reactions causing changes of the cellular biological system, that often result in close association with some pathologies. There is many evidence that links radicals to the aging processes, tumor and cardiovascular, neurodegenerative, infectious diseases[2-12]. On the other hand, another interesting aspect is the role played by antioxidants, mainly found in vegetable foods, which can counteract the toxic radical action. A large number of studies shows that the consumption of foods and drinks rich in antioxidants is often associated with a decreased risk of developing these diseases[13-19]. Taking into account these observations, it would seem appropriate to prescribe antioxidant drugs to prevent degenerative diseases or slow down the natural aging process. Moreover, diet could be enriched with products capable of defending the organism from the radicals. With our knowledge we cannot determine which substances are capable of having preventative or therapeutic effects, how long they have to be taken, nor the therapeutic dose. A class of antioxidant compounds (flavonoids and biophenols) are largely represented in vegetable and fruits, but there is still uncertainty on the beneficial effects of a diet rich in plant products.

Physiological and Pathological Role of Free Radicals

The term "free radicals" is usually associated to something dangerous and harmful to health or otherwise to avoid. This is supported by the fact that radicals are produced by radiation or environmental pollutants becoming radical, during their metabolism in our body. In fact, the free radicals are typically substances characterized by a high reactivity, that is responsible of their cytotoxic and genotoxic effects. However, less is known about the concept that some radicals are physiologically produced by the body (bio-radicals) and are essential to life. These are involved in enzymatic catalysis and other critical processes as, for example, the regulation of embryogenesis and the

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functioning of neuronal cells. The production and metabolism of free radicals, ROS and RNS in the human body are finely regulated processes, and an alteration of these mechanisms can lead to cellular injury as in chronical inflammatory state. In fact, the persistence of the inflammatory status is one of the major causes for the development of some types of cancer and neurodegenerative or cardiovascular diseases[20-22]. Bio-radicals play a major role in signaling pathways, modulating the cell's reduction-oxidation reactions (redox status) and, paradoxically, also playing an antioxidant action as "scavenger" of other radicals (radical-radical reactions). The importance of the cell redox status is due to its capability to modulate reversibly the transport, the metabolism, the fat accumulation, the activity of enzymes and transcription factors. In fact, many of these processes are regulated by redox centers (cysteines and metal-containing centers) whose oxidation or reduction, is responsible for biological events such as cell cycle, metabolism, neurotransmission, differentiation, cell-cell communication and apoptosis[23]. The excess of free radicals (oxidative stress), plays a pathogenic role: hyper-activation of some cellular signals (phosphorylation, transport), and the oxidation of specific protein sites (metal centers, SH, methionine, tyrosine, tryptophan, histidine) or DNA (guanine, xanthine, adenine). All of this leads to chronic inflammation, uncontrolled cellular proliferation, apoptosis or necrosis, resulting in cellular degeneration and a higher risk of developing cancer[24]. The complexity of cellular processes involving the oxidative stress suggests that the degenerative diseases and cancer are related to the excessive production of free radicals. A critical step is the understanding of the mechanisms that modulate the effects leading to the formation of chronic inflammatory process. Recently, several authors underlined the importance of two free radicals, anion superoxide ($\bullet O_2^-$) and nitric oxide ($\bullet NO$), carrying out essential functions as second messenger in the cells. These two radicals can react with each other to form a dangerous oxidizing species, peroxynitrite ($\bullet NO + \bullet O_2^- \rightarrow ONOO^-$)[25], that if produced in excessive quantities or in a compartment where the detoxification systems are unable to counteract their effects, can lead to a significant cellular injuries. The reaction between $\bullet O_2^-$ and $\bullet NO$ is a radical-radical reaction and its velocity is controlled by the diffusion and consequently, every time the two molecules meet produce peroxynitrite. Moreover, this reaction is faster than the superoxide reduction reaction carried out by the superoxide dismutase, the antioxidant enzyme able to counteract the excess of $\bullet O_2^-$. This implies that small amounts of peroxynitrite may form physiologically and probably be useful for the cell (for example in the redox signals)[26] and be metabolized by the tissue without causing cytotoxic reactions[27]. On the other hand, micromolar peroxynitrite levels produce sometimes irreversible protein and DNA oxidation, confusing the border between physiological and pathological activity not entirely clear[28]. In inflammatory neurodegenerative pathologies, for example, peroxynitrite probably plays an important cytotoxic role, as shown by the presence of 3-nitrothyrosine found in inflammatory or degenerative tissues[29,30]. In table I are shown the most common chemical modifications induced by an excess of free radicals that involve the macromolecules present in cells.

Assessment of Oxidative Stress in Man

As previously described, oxidative stress has been associated with a large number of diseases in humans, even if the relationship between the free radicals and some specific pathological processes has not definitively been established. In many pathologies, oxidative stress is not the primary cause of the disease, but a consequence of the cellular alteration caused by the disease. In order to protect themselves from oxidative damage, aerobic organisms, including humans, use a variety of antioxidant defense systems, both en-

Table I. Chemical modifications induced by oxidative stress.

Protein	Lipids	DNA
Carbonyl group	Hydroperoxides	8-hydroxyldeoxyguanosine
Hydroperoxides	Conjugated dienes	8-oxy-2-deoxyguanosine
Val and Leu Hydroperoxides	Isoprostane	8-hydroxylguanosine
3-nitrotyrosine	Malondialdehyde	5 hydroxylmethyluracil
2-oxo-histidine	4-hydroxylnonenal	8-oxyguanosine
Dityrosine	Total aldehydes	

Table II. Reduction potential of different antioxidants (pH 7.0, 20°C).

Antioxidant	Reduction potential
Ascorbate (Vitamin C)	282
Epigallocatechin	430
A-Tocopherol (Vitamin E)	500
Theaflavin	510
Caffeic acid	540
Epicatechin	570
Uric acid	590
Glutathione	920

dogenous and exogenous, strategically located in the various cellular districts. Inside the cells is possible to find specific enzymes that interact with ROS: superoxide dismutase, catalase and glutathione peroxidase, while most of membrane protection is due to the α-tocopherol, β-carotene and coenzyme Q. The antioxidant protection of extra- and intra-cellular fluids, is based on the action of metal binding proteins as ferritin, transferrin, ceruloplasmin, which retain metal ions. In particular, iron and copper in non-reactive forms, are able to prevent and to limit radical reactions. Moreover, the biological fluids are also protected by some important antioxidants such as vitamin E, uric acid, bilirubin, ascorbate and thiol groups[31]. It is, therefore, clear that the determination of the balance between pro-oxidant and antioxidant species can play an important role in the diagnosis and therapeutic treatment of certain pathologies related to oxidative stress[32]. A large number of methods has been proposed in the literature to measure oxidative stress or oxidative damage reaction products[33-35], notwithstanding free radicals are short life species, thus difficult to determine directly[36]. To overtake this, a complementary approach could be the measurement
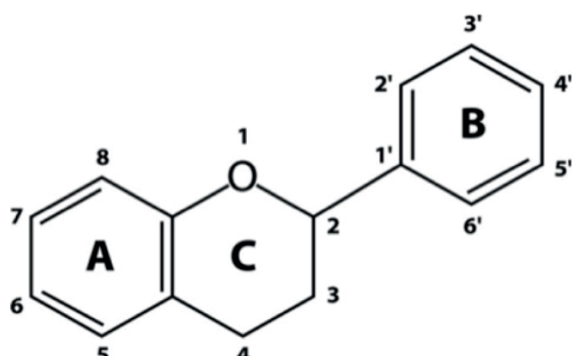
of the endogenous antioxidant defense systems, as a biological marker of oxidative damage. However, this approach is relatively simple to use in routine clinical biochemical analysis, but it presents some obstacles that prevent its current use. In fact, the scientific literature lacks in studies reporting the antioxidants plasma profile of patients affected by pathologies associated to oxidative stress, with respect to healthy people. Moreover, the depletion of endogenous antioxidants due to a particular diet, is not distinguishable from the one caused by the disease[37]. Consequently, the lack of universally accepted and standardized reference values for such measurements makes improbable, nowadays, the use of these biomarkers for clinical diagnosis. Another aspect to take into account is the "basal production" of oxidation products, that should be evaluated with care in order to differentiate it from the "pathological" ones. Likewise, we cannot rule out circadian variations of free radicals production.

Antioxidant Hypothesis

There are more than 5000 polyphenols in plant foods, most of which are flavonoids, including flavanols, flavonols, flavones, isoflavones, hydroxycinnamates and anthocyanins. These compounds are distributed among plants, fruits and vegetables in different amounts, all with different antioxidant activity. They represent the most abundant antioxidants in our diet; for example, in 200 g of fruits there are 500 mg of total polyphenols[38]. The antioxidant activity of polyphenols is related to their structure. In fact, it depends on the hydroxylation pattern, the position and the substitution of specific hydroxyl groups that differentiate the individual compounds[39]. Figure 1 shows the basic structure of flavonoids.

The following structures are important for antioxidant activity and detoxification of radicals: (1) the presence of two or three hydroxyl groups adjacent to ring B (catechol function at position 3' and 4' and pyrogallol function at position 3, 4' and 5', respectively); (2) the presence of other hydroxyl groups in the other rings (positions 5 and 7 of ring A, position 3 of ring C); (3) a double bond C2-C3 in ring C. Other hydroxyl groups may be present, for example, in position 5' of ring B and three more in positions 3', 4' and 5'of an esterified gallate group in position 3 of ring C[40]. The flavonoid structures can be very simple, such as caffeic acid, or complex, such as gallate epigallocatechin (Figure 2).

The reduction potential is a measure of the reactivity of an antioxidant, as a hydrogen or an

Figure 1. The basic structure of flavonoids.
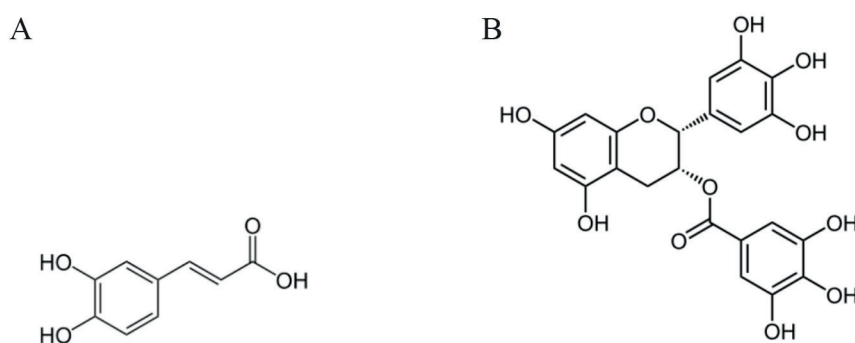
Figure 2. (*A)* Caffeic acid. (*B)* Gallate epigallocatechin.

electron donor, under standard conditions (Table II): a low reduction potential indicates greater capacity in hydrogen or electron donation, therefore reducing reactive species with unpaired electrons. Polyphenols generally have an average reduction potential in the natural antioxidant scale comparable to that of vitamin E[41], while ascorbic acid is considered the best natural antioxidant[42]. Polyphenols are able to detoxify most of the ROS and RNS, such as superoxide and hydroxyl radicals, hypochlorous acid, singlet oxygen, peroxyl radicals and peroxynitrite[43]. In addition, a major property tied mainly to the catechin structure is the ability to complex transition metals (copper and iron principally), preventing their catalytic activity in redox reactions[44,45]. Moreover, polyphenols also exhibit anti-inflammatory activity attributed to the inhibition of cyclooxygenase, lipoxygenase, myeloperoxidase, nitric oxide synthase and xanthine oxidase[46-49].

Factors that Contribute to the Different Biological Activities of polyphenols in vitro and in vivo

Although there is a huge amount of data on the antioxidant capacity *in vitro* of individual polyphenolic compounds in scientific literature, the efficacy of their consumption has not always been confirmed in clinical trials conducted on humans. In some cases, evidence of a protective effect was obtained[46,50-53], while in others it was not possible to correlate the efficacy of polyphenol consumption, with a decrease in oxidative stress markers (F2-isoprostanes, oxidized LDL, oxidation products of plasmatic proteins, damaged DNA)[54-57]. Moreover, some researches showed an increase in plasma protein oxidation after the consumption of fruit juice, supporting the fact that the mechanism of action of polyphenols is not completely clear[56,58]. It has also been observed that frequent consumption of drinks and foods (fruit juice and apples) rich in polyphenols, leads to a modest and transient increase in the total plasma antioxidant capacity. This could be explained by the increased levels of urate caused by fructose, contained in drinks and foods, rather than polyphenols[59,60]. The different biological activities shown *in vitro* and *in vivo* by polyphenols, are not only related to the heterogeneity of these molecules or to their structural and functional characteristics, but also reflect how these compounds are absorbed and metabolized in the gastrointestinal tract[61].

Transport and Absorption

The antioxidants' concentration in the stomach is comparable to that present in foods, while is not always predictable the amount of antioxidants actually available at the intestinal and hepatic level, because of the digestive processes happening there[62-64]. The absorption and the effects of polyphenols already begin at the level of the oral cavity[65,66], although greater absorption occurs in the gastric tract and, to a lesser extent, in the intestinal tract. After the consumption of 10 to 500 mg of polyphenols, the maximum plasma concentration generally does not exceed 1 μM, mainly due to poor absorption and metabolism by tissues and gastrointestinal microflora[67-69]. Consequently, polyphenols are less available than ascorbate and tocopherols, which have a specific absorption system. It was demonstrated that the antioxidant activity due to micromolar concentrations of polyphenols is significantly lower than that detected in plasma (>10^3 μM); in fact, at least 20-50 μM of polyphenols would be needed to effectively compete with endogenous antioxidants. *In vitro*, polyphenols and their metabolites, can decrease or inhibit the activity of some cell's membrane

transport systems, as demonstrated for glucose (GLUT2) and ascorbic acid (SVCT1), whose transport activity is inhibited by the presence of flavonoids[70]. The flavonoid glucosides, the most abundant polyphenol in the diet, can be transported to the enterocytes, via the sodium-dependent glucose transporter 1 (SGLT1) and metabolized to intracellular aglycones, by a β-glucosidase[71]. However, this pathway is less important because of the presence of an effluent intracellular glucose system directed towards the digestive tract lumen, through the Multidrug Resistance Associated Protein 2 (MRP2) transporter. In the lumen of the digestive tract, with the contribution of the intestinal bacterial flora, the flavonoids glucosides are definitively transformed into aglycones and absorbed from the intestinal and the blood cells through the MRP3[72].

Polyphenols Biotrasformation

The polyphenols undergo some significant biochemical modification in the oral cavity and gastrointestinal tract, before entering the circulatory system and reach the liver to be metabolized to several active metabolites. In fact, in saliva, the flavonoids glucosides are hydrolyzed to aglycones and then converted into compounds that can be absorbed by the epithelium of the oral cavity[73]. The reduction of polyphenols to monomeric units occurs mainly the stomach (Figure 3). In the small bowel and later in the liver, the two step transformation occurr: phase I, deglycosylation and formation of aglycones; phase II, transformation through the oxidative metabolism by the enzymes belonging to the P450 cytochrome family[74]. This last step leads to the formation of (a) methylated,

sulphate and glucuronidated products; (b) protein or thiols adduct (glutathione); (c) RNA or DNA adduct[75]. A further transformation takes place at large bowel level, where the intestinal microflora degrades flavonoids to simple phenolic acids, subsequently absorbed and metabolized in the liver, through the enterohepatic circulation. The methylated, sulphate and glucuronidated products continue to have biological activity[76], although lower with respect to the original molecule, because of the chemical modifications of the same (hydroxyl) groups responsible for the antioxidant activity.

Interactions and Synergies Between food Antioxidants and Endogenous Antioxidants

The interactions and synergies between food antioxidants and endogenous antioxidants are due to the redox potential of antioxidants (Table II). Antioxidants with lower redox potential are able to regenerate (reduce) other antioxidants, which have been previously oxidized into radicals. Regeneration reactions occur, for example, between polyphenols, ascorbic acid and vitamin E[77].

Gastric Cancer, Antioxidant Polyphenols, ROS and RNS

The highest concentration of polyphenols introduced with the diet can be found in the oropharyngeal tract, in the stomach, and partly in the intestine, before the absorption, metabolism or excretion process begins. In fact, after a diet rich in these antioxidant compounds, in the gastrointestinal tract can be detected μM concentrations of polyphenols, that could exert the protective effect against gastric and colorectal cancer.
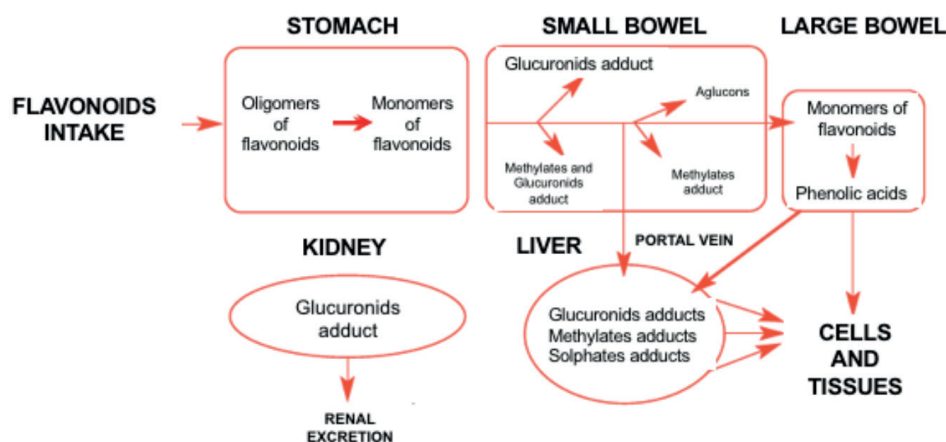

Figure 3. Metabolism of flavonoids.

Furthermore, the gastrointestinal tract is particularly exposed to the oxidative stress due to the ROS and RNS species from the diet and from the activation of the intestinal inflammatory cells[78]. In particular, RNS play an important role in the onset of stomach cancer, because in the presence of acidic pH they are able to form nitrating/nitrosating species that support the formation of carcinogenic nitrosamines[79]. Polyphenols have a direct protective effect on the gastrointestinal tract, detoxifying the ROS and RNS, preserving antioxidant proteins and complexing metals[80]. In the stomach the acid amplifies the peroxidation reactions catalyzed by the pro-oxidation compounds present in food, for example meat (i.e. Fe^{3+}-myoglobin, non-conjugated iron, fatty acids and hydroperoxides). These reactions are inhibited by catechin and red wine polyphenols, that in the gastric model were able to shift the reactions from pro-oxidants to antioxidant[81]. Similar results were obtained in other studies in conditions closer to physiological ones, where the polyphenols concentrations were comparable to those found in the stomach after a meal. All these results show that the polyphenols prevented the formation of nitrosamines involved in the pathogenesis of gastric cancer[82,83].

Conclusions

The available epidemiological data has shown a strong reverse correlation between the intake of fruits and vegetables and the occurrence of degenerative diseases and cancer. The observed effect, seems to be due to the synergistic action between the compounds of the endogenous antioxidant system (superoxide dismutase, glutathione peroxidase, glutathione-S-transferase and catalase) and the antioxidants from the diet. However, it is still unclear whether some foods are able to exert an optimal protective effect against cancer and much less we know about the hypothetical recommended levels of the antioxidants intake. Despite the interest in antioxidants as protective agents against cardiovascular, degenerative pathologies and cancer, the actual contribution of these compounds to the maintenance of health and their *in vivo* mechanism of action are not yet known. In fact, there is no substantial evidence that the *in vitro* antioxidant effect is the same that actually occurs *in vivo*. Flavonoids are poorly absorbed but are extensively metabolized in the gastrointestinal tract, mainly forming glucuronidated, sulphate and methylated conjugates, whose adducts are subsequently absorbed. Since the flavonoids and their metabolites are potent bioactive molecules able to interact with the intracellular signal pathways, it is mandatory to clarify their mechanisms of action such as antioxidants or signaling molecules, in order to assess their potential anti-tumoral role. More attention should be given to the effects of polyphenols in the gastrointestinal tract, where these compounds probably exhibit the highest antioxidant capacity, before they are metabolized and absorbed in the circulatory stream.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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