Antioxidant Activity of Natural Plant Extracts from the Perspective of their Effects against NO and its Oxidative Derivatives

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Abstract

The antioxidant activity of natural substances is mainly attributed to their phenolic content. However, biological effects mediated by potentiating or inhibiting Nitric oxide (NO) and its derivatives in organism are diverse and controversial. In this short review we discussed three different modes of action of selected compounds through production or scavenging of NO or its derivatives which have been identified in vitro conditions.

Keywords: Antioxidants; Nitric Oxide; NO Cycle; Peroxynitrite; Plant Extracts

Introduction

The biological activity of NO has gained much attention over the past decade as this molecule mediates a number of physiological processes, acting as a ubiquitous intracellular messenger in all vertebrates. The multiple physiological roles of NO includes regulation of vasomotor tone, cell adhesion to the endothelium, inhibition of platelet aggregation, and the proliferation of vascular smooth muscle [1]. Biological production of NO is also important for non-specific cellular immunity, although NO itself is not capable of killing intracellular pathogens or tumor cells. While it is often described as highly toxic and reactive, it has been demonstrated not to be so [2]. Nitric oxide interacts directly with a number of target molecules even before diffusing from the cells that produce it. The NO molecule is hydrophobic and readily crosses membranes. It exists for less than five seconds, but it diffuses from the site of synthesis to a distance of several cells, indicating that diffusion through the cell proceeds faster than the intracellular reaction of NO. Thus, this biomolecules interact in an autocrine and paracrine manner [3]. In circulating blood the half-life of NO is shorter, about 0.1 sec [4]. As NO and \( \text{O}_2 \) are nine times more soluble in a hydrophobic solvent, both tend to concentrate in hydrophobic parts of the cell [5]. The solubility of the reactants affects reaction rate and their concentration in membranes is ten times higher than that in the surrounding medium [5]. The reaction produces nitrates. The creation of reactive nitrogen is not inevitable. Nitric oxide is effectively scavenged by hemoglobin producing a nitrate and methemoglobin, which also prevents NO reacting with \( \cdot \text{O}_2^- \). The rate of binding of NO to the Fe (II)-hemoglobin is five to six times of that to \( \cdot \text{O}_2^- \). In contrast to the nitrite, the nitrate is biologically inactive, so NO oxidation in red blood cells is the most efficient and definitive pathway of NO inactivation [6].

In the vicinity of NO, the synthesized superoxide anion radical (\( \cdot \text{O}_2^- \)) in cell will spontaneously combine to form peroxynitrite (\( \text{ONO}_2^- \)) in a diffusion-controlled reaction [7]. The reaction continues without enzyme catalysis, as no enzyme involving in this reaction. Nitric oxide is one of the biological molecules known to react rapidly with superoxide and produces “knock-out” competition for the superoxide dismutase substrate. Therefore, peroxynitrite formation is an essential part of the ongoing processes, which forms by reaction of NO and \( \cdot \text{O}_2^- \) in vivo [2]. In the literature, NO is most frequently associated with the physiological role of signal substances, regardless of the complexity and certainly controversial events that are raised.

NO production requires two oxygen atoms. As the half-life of NO is 7 sec, 120nmol of \( \text{O}_2 \) per g of tissue would be necessary per minute to maintain the steady state NO concentration of 1µM. The cytotoxic effects may be seen at submicromolar concentrations, at which NO reversibly inhibits metalloenzymes such as catalase and cytochrome P-450 [8]. Ribonucleotide reductase inhibition is beneficial in inhibiting the replication of viruses, but its activity is restored at lower levels of NO. Therefore, to maintain the replication inhibition, constant increased production of NO is needed. This occurs only in the main phase of inflammation or near macrophages. Cytochrome c is also reversibly inhibited at submicromolar concentrations [9], which can temporarily lead to an increased release of \( \cdot \text{O}_2^- \) in the electron transport chain. This can then combine with NO, which results in formation of \( \text{ONO}_2^- \) causing irreversible damage to mitochondria. Nitrosative stress refers to the conditions under which the amount of NO is sufficient for the nitrosation of both thiols and amines through a nitrosative mechanism (formation of nitrosated species) or oxidative mechanism (oxidative nitrosylation). Thiol modification leads to changes in their activity and functionality. Nitrosation of amines leads to the formation of carcinogenic nitrosamines or deamination [10]. However, NO also plays an important role in radical chain reaction termination, for example in the conversion of thiol radicals to nitrosothiol, thereby terminates the radical reactions [2]. It reacts with the reactive forms such as an alkyl, epoxyallyl, alkoxy or peroxyl radicals to form unstable products of radical reaction termination products [11]. Basically, nitrosative stress can be seen in many cases as antioxidant activity. Many biological effects attributed to reactive nitrogen species are in fact mediated by \( \text{ONO}_2^- \). The elevation of both \( \cdot \text{O}_2^- \) and NO by a factor of 10, would result in a 100-fold increase in the formation of \( \text{ONO}_2^- \). Biological and pathological consequences in the presence of \( \text{ONO}_2^- \) are interesting as they relate to specific effects.

Determination of the concentration of NO decomposition products such as nitrite and nitrate certainly provides a reasonable approximation of the level of NO in vivo. It is necessary to provide an analysis of the conditions of disease state that would results in determining appropriate solutions for elimination of reactive species. Extrapolation of in vitro to the in vivo situation may provide a provisional indication of the concentration of NO as well as other reactive species.

It has been found that micromolar concentrations of vitamin E and many polyphenols are effective against oxidative stress through direct scavenging of reactive oxygen species as shown in...
the cell culture model for the study of cancer, atherosclerosis, and neurodegenerative diseases [12-14]. Clinical studies and studies on primary prevention of high doses of antioxidants in humans, however, have not produced any benefit [15]. Nevertheless, epidemiological data on health benefits for their consumption are dear [16]. In normal consumption, relatively low amounts of these phytochemicals enter the body to achieve the direct antioxidant (micromolar) concentrations in cells. According to Jablonowska-Lietz et al. [17], total daily amount of vegetables and fruit consumed by adults is 513 g. It is also worthwhile noting that apart from antioxidant vitamins, fruit and vegetables contain other substances documented as being beneficial to health, including dietary fibre, flavonoids and minerals. However, the average daily intake of antioxidant vitamins consumed by the study participants ostensibly fulfill dietary requirements. It is therefore still remains of considerable interest to find the mechanisms of action of commonly consumed low (submicromolar) concentrations. In in vitro studies, the activity of a number of selected plant extracts known for their variety and positive health effects were tested against reactive oxygen and nitrogen species. Siberian ginseng (Eleutherococcus senticosus (Rupr. & Maxim.) Maxim) exhibited very good scavenging properties against ·O₂⁻ and also ·HO. Furthermore, stevia (Stevia rebaudiana (Bert.)) has also manifested notable characteristics toward ·HO. Interestingly, these two extracts were also capable of reducing the level of NO oxidative products. By comparison, sage (Salvia officinalis (L.)) and oregano (Oregano vulgare (L.)) did not show such effects and could influence theredox state of the cells to the extent of affecting the viability of the tested tumor cell lines, this was particularly clear in the case of oregano. In summary, the results obtained indicate that the oregano extracts achieve a good potency level at concentration of 100μg/ml, and to a lesser extent [18]. Our findings can provide direction for further research on the targeted delivery of extracts of Siberian ginseng and stevia in diseases arising from or caused by oxidative stress. In addition, the described properties of oregano and sage could also be used. Conversely, the utility of acquired characteristics is normally guaranteed by a low dose that is taken up by the body, which is less than the range tested as the extract palatability formed a natural barrier. Relatively low doses are thus able to activate cellular stress response without causing adverse effects on their function. More specifically, this form of confinement addresses the described concept of stabilization or adaptive stress response. Recent findings suggest that many health benefits of phytochemicals occur through cross-modal hermetic mechanisms which activate one or more of the adaptive pathways of the cellular stress response [19].

Other compounds with described healing properties, such as dry horse chestnut (Aesculus hippocastanum (L.)) extract and its main components in, provide a completely different view on biological activity in the body as their basic action in the organism is connected with the stimulation of NO synthesis. An important finding is that the ability to convert nitrite declined with increasing concentration of the horse chestnut extract, almost disappearing. Escin extract acted in a similar manner but less strongly. At lower concentrations (lower than 25μg/ml), an increase in peroxynitrite levels was caused again [20]. Moreover, the overall activity against ·O₂⁻ at blood pH levels was not significant. The induction of Nitric Oxide Synthase (NOS) activity in the body under certain conditions can lead not only to increased NO production, but also to the formation of ·O₂⁻ [21]. The increased formation of ·O₂⁻ can occur under inflammatory conditions. This can very easily lead to increased peroxynitrite levels in the body by the described mechanism and with respect to the activity of extracts from our findings. There is essentially no scavenging activity against this reactive form. In fact, to the contrary, low-concentration escin can increase the level of peroxynitrite. An increase of peroxynitrite itself is a prerequisite for the partial uncoupling of NOS activity and increased formation of ·O₂⁻ and NO. Cyclical prevalence of NO or ONO₂⁻ produces the so-called NO cycloin which increases levels ONO₂⁻ and then cyclically activates nuclear factor kappaB (nuclear factor kappa-light-chain-enhancer of activated B cells, NF-kB) [21]. Such effects can cause adverse effects or result in the chronic progression of the disease in the body (such as chronic fatigue syndrome, myalgic encephalomyelitis, multiple chemical sensitivity, fibromyalgia, large number of other chronic inflammatory diseases and posttraumatic stress disorder). The ability to modulate the production and the uptake of the NO oxidative product, including scavenging ability towards superoxide radicals, makes it necessary to evaluate sufficiently high individual doses carefully to demonstrate the therapeutic effects of extracts. The question is to ingest a sufficient individual dose (80μg/ml) in order to strengthen the body and avoid the need for treatment. On the other hand, it is somewhat agreeable that horse chestnut and escin do not form a normal part of the diet, even in small quantities.

The third and another different situation is the extrapolation of the in vitro activity of the substances taken up by the organism daily and in relatively higher amounts in proportion to the above mentioned. It may be encouraging that, while monitoring the activity of salicylic acid and its three dihydroxy derivatives, it was found that the activity against NO oxidative products in the blood pH is slightly higher than that against the ·O₂⁻. This is not the case, however, for the most common catechin metabolite in the body, protocatechuic acid, which maintains higher levels of NO, as shown by our initial study [22]. Taking into account the uniqueness of individuals, vasodilation caused by the emitted NO may lead to an immediate headache. The perceived effects of the protocatechuic acid (3,4-dihydroxybenzoic acid) may therefore not be so beneficial in general and may cause side effects in the form of abdominal pain or headache.

Due to the ever growing trend of using natural substances as active antioxidants in addition to drugs to achieve and maintain a normal status of a healthy individual, the extrapolation of some experiments could explain their effect on the body. This could be useful in their application in disease states related to or contingent on oxidative stress or simply to induce a hormetic response. The view of plant extracts as antioxidants essentially differs depending on whether we only know their effect against reactive oxygen species or also on reactive nitrogen species. The ability to change the amount of NO and its oxidative derivatives can fundamentally change the nature of their action on the body.

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References


