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Differentiation between stoichiometric and anticatalytic antioxidant properties of benzoic acid analogues: A structure/redox potential relationship study

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ABSTRACT

We investigated the antioxidant activities of some phenolic acid derivatives on a cell free system and on cellular and enzymatic models involved in inflammation. The stoichiometric antioxidant activities of phenolic acid derivatives were studied by measuring their capacity to scavenge the radical cation 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$) and reactive oxygen species (ROS) produced by stimulated neutrophils. The anticatalytic antioxidant capacity of the molecules was evaluated on the activity of myeloperoxidase (MPO), an oxidant enzyme present in and released by the primary granules of neutrophils. The ROS produced by PMA-stimulated neutrophils were measured by lucigenin-enhanced chemiluminescence (CL) and the potential interaction of the molecules with MPO was investigated without interferences due to medium by Specific Immuno-Extraction Followed by Enzyme Detection (SIEFED). The antioxidant activities of the phenolic compounds were correlated to their redox potentials measured by differential pulse voltammetry (DPV), and discussed in relation to their molecular structure.

The ability of the phenolic molecules to scavenge ABTS radicals and ROS derived from neutrophils was inversely correlated to their increased redox potential. The number of hydroxyl groups (three) and their position (catechol) were essential for their efficacy as stoichiometric antioxidants or scavengers. On MPO activity, the inhibitory capacity of the molecules was not really correlated with their redox potential. Likewise, for the inhibition of MPO activity the number of OH groups and mainly the elongation of the carboxylic group were essential, probably by facilitating the interaction with the active site or the structure of the enzyme. The redox potential measurement, combined with ABTS and CL techniques, seems to be a good technique to select stoichiometric antioxidants but not anticatalytic ones, as seen for MPO, what rather involves a direct interaction with the enzyme.

1. Introduction

Phenolic acids are aromatic secondary metabolites widely spread through the plant kingdom and involved in plant life protection [22,42]. These compounds are ubiquitous in plant food (i.e. fruits, vegetables, leaves) and therefore a certain quantity of them is consumed in our daily diet [22,35]. The basic skeleton of phenolic acids remains the same, but the number of the hydroxyl groups on the aromatic ring and the modifications of the carboxylic acid function create the variety of the molecules (see the structures of the molecules in Fig. 1). The pharmacological functionality of phenolic acid derivatives has attracted much attention due to their antioxidant, anti-inflammatory, anti-fungal, anti-bacterial and antimutagenic properties that can be exploited in health [28,35,36,46]. One of the vast areas of interest of phenolic acids has been in food quality and preservation. In plants, flavonoids are the predominant phenolic class described which account for approximately the two-third of the dietary phenols. Phenolic acids account for almost all of the remaining third and there is an increasing interest in the antioxidant behaviour and potential health benefits associated with these simple phenolic acids [42] especially on the inflammatory response. Plasma concentration of an individual phenolic acid is dependent of the dietary intake and rarely exceeds 1 μM [34]. However, there is an evidence for microbial origin of some phenolic acids in blood and the total

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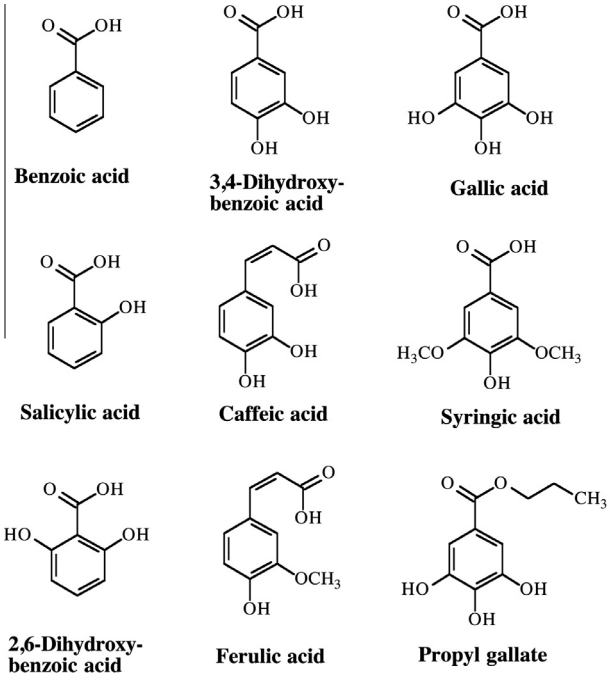

Fig. 1. Chemical structure of the benzoic acid analogues studied.

concentration could vary from 5 μM in the norm to 55 μM in sepsis that could modulate the production of reactive oxygen species (ROS) by neutrophils and mitochondria [3,10].

Polymorphonuclear neutrophils (PMNs) play key roles in the immunological and inflammatory responses. PMNs are specialised for their primary function of phagocytosis, with highly developed mechanisms for intracellular digestion of particles, such as pathogens and cell debris. However, excessive activation of PMNs generates ROS and discharges hydrolytic and proteolytic enzymes, all implicated in several human and animal diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, sepsis, cataracts, DNA damage and inflammation, etc. [2,25,48]. Myeloperoxidase (MPO), a specific granular enzyme of PMN, is considered as a marker of stimulated PMNs and contributes to oxidative stress by generating oxidant species. MPO has a dual activity of chlorination and peroxidase [26]. From its chlorination activity the enzyme leads to the formation of hypochlorous acid (HOCl), a highly oxidant compound, responsible for the microbial killing and the oxidation and chlorination of proteins [7]. Through its peroxidase activity, MPO could lead to the formation of oxidant species such as nitrogen dioxide ($^{\cdot}NO_2$) involved in protein nitration [18]. In some conditions involving acute and chronic inflammation, MPO is released in the extracellular medium by highly stimulated and dying neutrophils and is able to exert oxidant activity on neighbouring cells and tissues. Thus, inhibiting the enzymatic activity of MPO or decreasing the oxidant activity of PMNs may prove beneficial for the treatment of inflammation-related diseases [7,16,30].

There is a growing interest in the therapeutic or nutritional use of natural substances for their anti-cancer, anti-atherosclerosis, anti-inflammatory and antioxidant properties. Antioxidant molecules act by trapping reactive oxygen species (ROS) (stoichiometric activity), or by inhibiting the enzymes and pathways responsible for the production of ROS (anticatalytic activity). It is evident that the anticatalytic activity is more powerful than the stoichiometric one, because low concentrations of anticatalytic antioxidant molecules are sufficient to block or reduce an increased ROS production. In the present study, we investigated the antioxidant capacity of phenolic acid derivatives by measuring their capacity to quench the radical cation 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\cdot+}$) and evaluated their anti-inflammatory like properties via their ability to modulate the oxidant response of neutrophils and myeloperoxidase activity. The antioxidant and anti-inflammatory activities of the phenolic compounds were studied based on their respective oxydo-reduction potentials determined by differential pulse voltammetry (DPV) by using a voltammetric home made device for small volumes (Fig. 2). The licenced immunological technique SIEFED (Specific Immuno-Extraction Followed by Enzyme Detection) developed to measure specifically active MPO in complex fluids [12] was used as a pharmacological tool to screen inhibitors having an interaction with the enzyme (Fig. 3). All the results were discussed in terms of molecular structure and redox potential relationship.

2. Material and methods

2.1. Chemicals and reagents

Phosphate salts, sodium, potassium and calcium chlorides, Tween 20, Trypan blue, H_2O_2 are from VWRI (Leuven, Belgium). 2,2′-Azinobis-(3-ethylbenzohiazoline-6-sulphonic acid (ABTS) was from Fluka (Bornem, Belgium). Amplex Red, EDTA, DMSO, Percoll, sodium persulfate ($Na_2S_2O_8$), sodium nitrite, lucigenin, methanol (MeOH), phorbol 12-myristate 13-acetate (PMA), benzoic acid, 2,6-dihydroxy-benzoic acid, gallic acid (2,4,6-trihydroxybenzoic acid), 3,4-dihydroxy-benzoic acid, salicylic acid (2-hydroxy-benzoic acid), caffeic acid [3-(3,4-dihydroxyphenyl)-2-propenoic

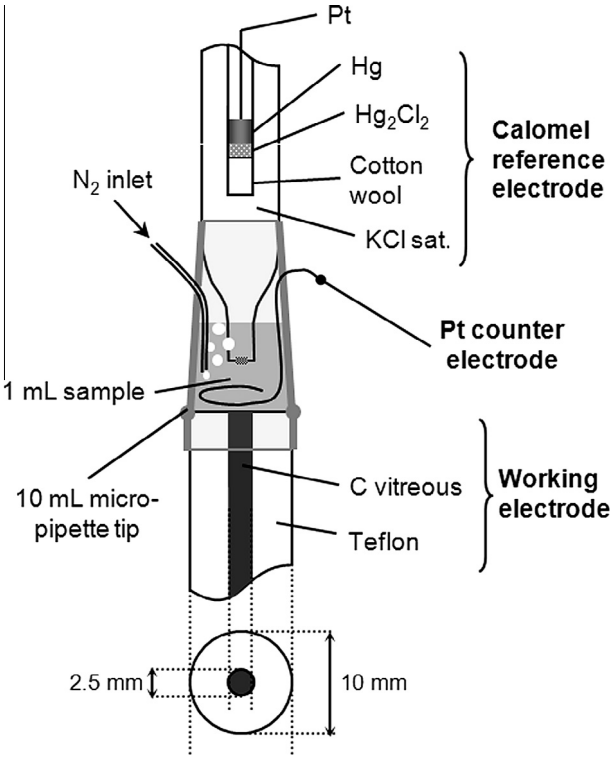

Fig. 2. Schematic representation of homemade device designed to allow electrochemical measurement in small samples under inert atmosphere. Top-part: universal saturated calomel reference electrode (SCE). Mid-part (in grey): the homemade plastic cell, formed by a 10 mL tip cut to fit the diameter of the calomel electrode, equipped of N_2 inlet tube and platinum wire counter-electrode. Bottom-part: Working electrode composed of a vitreous carbon cylinder (ø = 2.5 mm) embedded in Teflon tube (ø = 10 mm).