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Coumarin Derivatives and Oxidative Stress

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Abstract: Coumarins, an old class of natural and synthetic benzopyrene derivatives, attract an intense interest because of their diverse pharmacological use. The biochemical properties and therapeutic applications of simple coumarins strongly depend upon the molecular structure of these compounds. Recently their antioxidant effects were extensively examined in connection with possibilities for control over the oxidative stress. Latter is being implicated in many diseases curable by therapy with coumarin-containing drugs. In this review, experimental data for antioxidant properties of coumarins are presented. It was observed that different coumarins may act as antioxidants by scavenging free radicals, by acting as enzymes modulators and by bonding free metal cations in helate complexes. The antioxidant activities are discussed in relation with their molecular structure.

Key words: Synthetic and natural coumarins, antioxidants

INTRODUCTION

The balance between formation and elimination of free radicals determines the overall stability of a living body[1-3]. Free radicals originate from large variety of normal and pathological metabolic transformations, from host-defense against undesirable invasion (chemical or biological) and from host-response to a disturbance of the tissues' integrity (do to trauma, cellular damage, etc). Free-radical chain reactions in the body are initiated mostly by Reactive Species (RS-molecules, ions, free-radicals), possessing oxygen (ROS) or nitrogen (RNS) atom with unpaired electron[1-5]. If more RS formed than needed for the normal redox-signaling and self-defense of the host, oxidative stress (OS) occurs leading to an oxidative cellular damage, even to cellular death (Fig. 1). If developed locally in the place of the invasion or trauma, the oxidative stress is part the healing process. The oxidative damage of the host's tissues is restrained by the endogenous antioxidant defense. In case of severe OS or insufficient endogenous antioxidant defense, severe host's tissue damages may occur.

Free-radicals induce the cell damage by altering the biological activities of lipids, proteins, DNA and carbohydrates (Fig. 1a). Damaged cells function incorrectly. It results to a further escalation of the oxidative stress and to an enhanced tissue damage. Simultaneous ionic disbalance, mitochondrial dysfunction and activation of the caspase/calpine cascades, result in a cell death (Fig. 1b). The negative effects of the RS may be diminished by limiting their synthesis (control over metabolic transformations and enzymes producing RS), by recombination of the RS already formed (e.g., "radicals scavenging") and by altering the RS-effects[2-4]. Inhibitors of the RS synthesis (NADPH-oxidases, XO-inhibitors; leukocytes' antibodies) may be administered. Therapeutics which intensify or supply the enzyme systems involved in the antioxidant defense (SOD, CAT, GSX) can be applied. The iron and copper metabolism may be affected by using chelators and drugs stimulating the binding ability of Fe and Cu binding proteins. Some substances directly recombine free radicals and this way interrupt the initiation and/or propagation of the free-radicals induced chain reactions.

The large variety of possible substitutions in the basic molecule (1) may influence the structure-related biological activities of coumarins. These compounds play an important role in plant biochemistry and physiology. They act as antioxidants, enzyme inhibitors and precursors of toxic substances. They are involved in the actions of plant growth hormones and growth regulators, the control over the respiration, photosynthesis, as well as in the defense against infection. The coumarins have long been recognized to possess anti-inflammatory, antiallergic, hepatoprotective, spazmolitic, antithrombotic, antiviral and anticarcinogenic activities. As oxidative stress is implicated in development of many pathologies (1), one possible aspect of the therapeutic effect of coumarins might be related with their antioxidant action (Table 1). The hydroxycoumarins are typical phenolic

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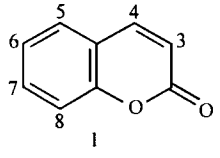
Table 1: Structures of coumarins used as antioxidants

Compound		R3	R4	R5	R6	R7	R8
1	Coumarin	H	H	H	H	H	H
2	7-hydroxycoumarin	H	H	H	H	OH	H
3	7-methylcoumarin	H	H	H	H	Me	H
4	4-methylcoumarin	H	Me	H	H	H	H
5	4-methyl-7-hydroxycoumarin	H	Me	H	H	OH	H
6	3-hydroxyscopoletin	OH	H	H	OMe	OH	H
7	3,7-dihydroxycoumarin	OH	H	H	H	OH	H
8	4-methyl-6,7-dihydroxycoumarin	H	Me	H	OH	OH	H
9	7,8-dihydroxy-4-methylcoumarin	H	Me	H	H	OH	OH
10	6,7-dihydroxycoumarin (Esculetin)	H	H	H	OH	OH	H
11	Esculin	H	H	H	OGl	OH	H
12	4-hydroxycoumarin	H	OH	H	H	H	H
13	7,8-diacetoxy-4-methylcoumarin	H	Me	H	H	acetoxy	acetoxy
14	7,8-dihydroxy-6-methoxycoumarin	H	H	H	OMe	OH	OH
15	3-hydroxycoumarin	OH	H	H	H	H	H
16	3-aminocoumarin	NH_2	H	H	H	H	H
17	3-acetylaminocoumarin	acetylamino	H	H	H	H	H
18	Coumarin-3-carboxylic acid	COOH	H	H	H	H	H



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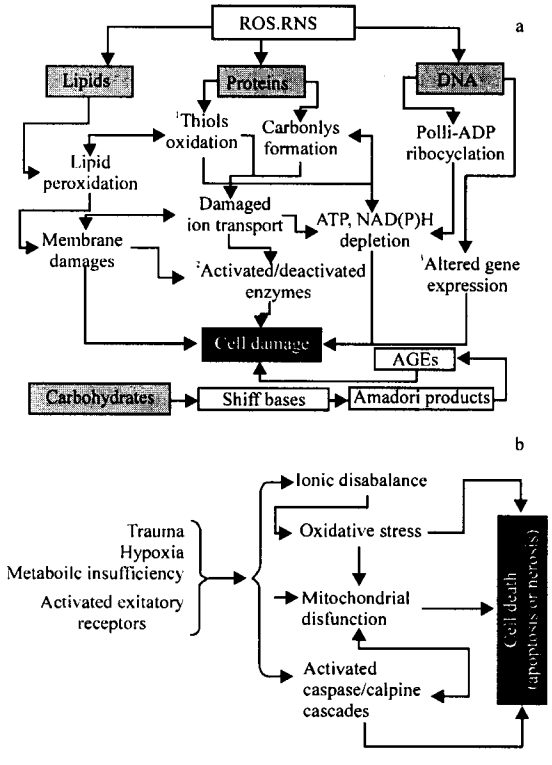

Fig. 1: Implication of the Reactive Species (ROS and RNS) and of the Oxidative stress (OS) in the cell damage (a) and in the cell death (b)

compounds and therefore, act as potent metal chelators and/or free radical scavengers, which may result in a powerful antioxidant effect.

Coumarin (1) itself has a low absolute availability in the human body due to first-pass hepatic catalytic conversion (in presence of Citochrome P-450) into 7-hydroxycoumarin (2), followed by glycoronidation[6]. In general, coumarin is being involved in the DNA repairing (by acting as a antimutagen)[7-9], in the restoration of damaged SOD, CAT, GPX activities, activation of other enzymes which participate in the immune defense (quinone oxidoreductase, glutathione-S-transferase, glutathione synthetase)[10-12] and in depression of the Xanthine Oxidase-activity[10]. Decreased MDA, LPO and DNA damaged were found after using coumarin[7-12]. By decreasing the activity of the Xanthine oxidase (XO)[10], coumarin is capable to decrease the level of $^{\cdot}O_2$-formation, diminishing the probability for cellular damage through all pathways presented in Fig. 1. In addition, it is able to reduce the cellular damage independently through pathways 1, 2 and 3 shown in Fig. 1a.

The antioxidant properties of coumarin derivatives are of various nature. Coumarins recombine radicals which initiate or propagate lipid peroxidation (act as radicals' scavengers), deactivate some enzymes and form chelate complexes with some metal ions (Fe, Cu) being involved in the free radicals-formation[6,13,14]. To show antioxidant activity, a coumarin derivative has to possess at least one hydroxyl group[13-15].

The antioxidant properties of monohydroxycoumarins have been related with radicals-scavenging activity, inhibition of tyrosine kinases and possibly, with effect on the prostaglandin proteination[6,15]. The antioxidant activity enhances if the OH group is situated on the flavonoid ring, while being depressed after substitution of H with metoxy (-OCH_3) or methyl (-CH_3)[13,16] in the molecule. For example, 7-methylcoumarin (3) is antioxidant[15], 4-methylcoumarin (4) is prooxidant[13], while 7-hydroxy-4-methylcoumarin (5) acts either as antioxidant or as prooxidant depending on the source of free radicals[13]. If the OH group is connected to a C3 atom (such as 3-hydroxyscopoletine (6) and 3-hydroxyumbelliferone (7)), the corresponding coumarin is inhibitor of 5-lipoxygenase and α-D-glucosidase[14].

Dihydroxycoumarins are better antioxidants than monohydroxycoumarins[6,13,17]. The OH groups positioned near C6 and C7 in the coumarin skeleton play an important role in the inhibition of the mushroom tyrozinase[17]. The 6,7-dihydroxy-4-methylcoumarin (8) is free radicals' scavenger too[13]. The most promising antioxidant properties showed 7,8-dihydroxy-4-methylcoumarin (9)[13]. If the two OH groups are in ortho-positions, the corresponding dihydroxycoumarine can decrease the lipid peroxidation by scavenging $^{\cdot}O_2$-and ROO$^{\cdot}$, but in presence of free Fe^{3+} this compound helps the $^{\cdot}$OH-generation and thus behave as prooxidant[6]. The effects of coumarin and its derivatives on rat platelet lipoxygenase and cyclooxygenase activities were studied by Sekiya *et al.*[18]. Esculetin (6,7-dihydroxycoumarin) (10) was found to inhibit the lipoxygenase more strongly than the cyclooxygenase; its concentration for 50% inhibition (IC_{50}) was 0.65 microM for platelet lipoxygenase and 0.45 mM for platelet cyclooxygenase. Esculin (the 6-glucoside of esculetin) (11) and umbelliferone (7-hydroxycoumarin) also selectively inhibited the lipoxygenase, though less strongly (IC_{50} = 290 and 500 mM, respectively). The mechanism of the lipoxygenase inhibition by esculetin was non-competitive. 4-Hydroxycoumarin (12) and coumarin had no inhibitory effect on either enzyme at concentrations up to 1 mM. Other antioxidants (hydroquinone, gallic acid and ascorbic acid) were less inhibitory to both enzymes and showed little selectivity. This investigation might suggest involvement of OH group near C7 and lipoxygenase inhibitory activity of dihydroxycoumarins. This activity is enhanced by the second OH group near C6 and may be altered by involving the latter in substitution reactions. Twenty-three 4-methylcoumarins bearing different functionalities have been examined by Raj *et al.*[19-21] for the first time for their effect on NADPH-catalysed liver-microsomal lipid peroxidation with a view to establish structure-activity relationship. Unlike 4-methylcoumarin which is prooxidant[13], Dihydroxy-and diacetoxy-4-methylcoumarins produced dramatic inhibition of lipid peroxidation. 7,8-Diacetoxy-4-methylcoumarin (13) and 7,8-dihydroxy-4-methylcoumarin were found to possess superb antioxidant and radical scavenging activities. Fraxetin (14) prevents oxidative stress by an important increase in antioxidant reserves of GSH and peroxidative damage is preserved by fraxetin treatment[22].

The effectiveness of the antioxidant action against metal ion-induced peroxidation is determined by the capability of coumarins to chelate transition metals' cations (mainly free Fe and Cu)[16,23]. The antioxidant activity of Cu^{2+}-helated coumarins is being suggested to be similar to this of the Cu-Mn-Superoxide Dismutase[23]. The antioxidant activity increases if the OH group is positioned in the flavonoid ring and decreases if metoxy group appears in the molecule[16].

The antioxidant capacity of 3-hydroxycoumarin (15), 3-aminocoumarin (16), 3-acetylaminocoumarin (17) and 3-coumarin carbonic acid (18), has been investigated with chemiluminescence measurement and by the accumulation of TBA-active products[24]. All these coumarins were found to be antioxidants, which suggests the importance of the functional group near the C3 atom for the decrease of the MDA formation.

Thus, the oxidative stress is important factor for the host's self-defense. As the oxidative attack is not specific to the origin of its targets, it results in a cellular damage of guest and host altogether. The extent of the host's cellular damage depends on the efficacy of its endogenous antioxidant defense. Latter may be affected by therapeutical control over the main illness of the patient. Coumarin and its derivatives are widely used in the medical practice. The oxidative behavior of these compounds is strongly determined by all aspects of their molecular structure. Depending on their structure and on the source of the oxidative stress, coumarins might act as antioxidants or prooxidants. Their administration along with vitamins and other antioxidants has to be considered taking into account the oxidative behavior of the particular coumarin derivative and the nesessity of oxidative stress for the healing process.

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