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In vitro total antioxidant capacity and anti-inflammatory activity of three common oat-derived avenanthramides

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ABSTRACT

To better understand mechanisms underlying the health benefits of oats, the free radical scavenging capacities of oat avenanthramides 2c, 2f, and 2p and their ability to inhibit NF-κB activation were evaluated. The antioxidant capacities of 2c, 2f, and 2p against peroxyl radicals, hydroxyl radicals, superoxide anion, singlet oxygen, and peroxynitrite were determined by using ORAC, HORAC, SORAC, SOAC, and NORAC assays, respectively. The total antioxidant capacity of 2c was approximately 1.5-fold those of 2f and 2p. Total antioxidant capacity was primarily attributable to SORAC and ORAC for 2c (>77%, $p < 0.05$), and to ORAC and SOAC for 2f. ORAC accounted for approximately 32% of total antioxidant capacity in 2p. EC_{50} values for inhibiting TNF-α-induced NF-κB activation in C2C12 cells were 64.3, 29.3, and 9.10 μM for 2c, 2f, and 2p, respectively. Differences in antioxidant capacities and ability to inhibit NF-κB among the avenanthramides could be ascribed to structural variations.

1. Introduction

More than 25 avenanthramide compounds in oats have been identified, although some of these have not been completely characterized. Structurally, all avenanthramides contain an anthranilic acid and a cinnamic acid, but the substitution patterns on the anthranilic acid and cinnamic acid distinguish them from each other (Bratt, Sunnerheim, Bryngelsson, Fagerlund, et al., 2003). The most abundant avenanthramides in oats are N-(3′,4′-dihydroxy-(E)-cinnamoyl)-5-hydroxyanthranilic acid (2c), N-(4′-hydroxy-3′-methoxy-(E)-cinnamoyl)-5-hydroxyanthranilic acid (2f), and N-(4′-hydroxy-(E)-cinnamoyl)-5-hydroxyanthranilic acid (2p) (Fig. 1). These compounds differ by a hydroxyl group, methoxy group, or hydrogen on the 3′ carbon of the cinnamic acid (B) ring, which may account for differences in biological effects.

A large body of evidence suggests the involvement of ROS and RNS, including $ROO^{\cdot}$, $HO^{\cdot}$, $O_2^{\cdot-}$, $^1O_2$, and $ONOO^-$ in the pathophysiology of aging and in chronic diseases (Finkel & Holbrook, 2000). In addition, free radicals can undergo interconversion. For example, $O_2^{\cdot-}$ is converted to oxygen and H_2O_2 by superoxide dismutase, $O_2^{\cdot-}$ also reacts with nitric oxide ($NO^{\cdot}$) to form $ONOO^-$, a more biologically damaging radical than either of the reactants (Beckman & Koppenol, 1996). In living cells, endogenous and exogenous antioxidants can neutralize and/or prevent the damage caused by ROS/RNS. These antioxidants include avenanthramides, readily bioavailable polyphenols in oats that have been shown to possess numerous beneficial properties, including anti-inflammatory, antiatherogenic, antiproliferative, anticancer, and anti-itch effects that may be useful in prevention of coronary heart disease, colon cancer, and skin irritation. These low-molecular-weight, soluble phenolic compounds are constitutive components of oat groats (Collins, 1986), hulls, bran (Emmons & Peterson, 2001), and leaves (Peterson & Dimberg, 2008), reaching a total level of from approximately 2 to 300 mg/kg in oat grains.

Abbreviations: AUC, area under the curve; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EC_{50}, half maximal effective concentration; C, (+)-catechin; GC, (+)-gallocatechin; EC, (−)-epicatechin; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; EGCG, epigallocatechin gallate; $HO^{\cdot}$, hydroxyl radicals; HORAC, hydroxyl radical absorbance capacity; NORAC, peroxynitrite radical absorbance capacity; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IκB, inhibitor of NF-κB; $NO^{\cdot}$, nitric oxide; ROS, reactive oxygen species; RNS, reactive nitrogen species; $ROO^{\cdot}$, peroxyl radicals; $O_2^{\cdot-}$, superoxide anion; $^1O_2$, singlet oxygen; $ONOO^-$, peroxynitrite; ORAC, oxygen radical absorbance capacity; SOAC, singlet oxygen absorbance capacity; SORAC, superoxide radical absorbance capacity; TE, Trolox equivalent; TNF-α, tumor necrosis factor-alpha.

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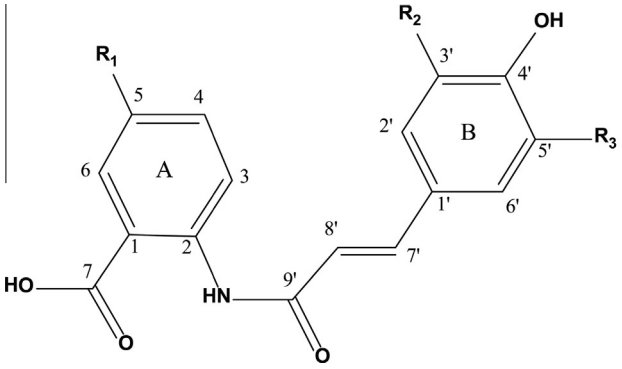

Avn	R_1	R_2	R_3
1c	H	OH	H
1p	H	H	H
1s	H	OCH_3	OCH_3
1f	H	OCH_3	H
2c	OH	OH	H
2f	OH	OCH_3	H
2p	OH	H	H
2s	OH	OCH_3	OCH_3

Fig. 1. Chemical structures of oat avenanthramides.

In vitro antioxidant activities of avenanthramides have been extensively studied (Bratt et al., 2003; Chu et al., 2013; Dimberg, Theander, & Lingnert, 1993; Peterson, Hahn, & Emmons, 2002) and found to be 10–30 times those of other phenolic antioxidants in oats (e.g., vanillin, caffeic acid). The avenanthramides 2c, 2p and 2f demonstrated antioxidant activity in the DPPH and β-carotene bleaching assays, with 2c showing the strongest antioxidant capacity of the three compounds (Peterson et al., 2002). In a linoleic acid model system, 2f displayed an antioxidant capacity approximately 20% that of α-tocopherol (Dimberg et al., 1993). In another study, all eight avenanthramides tested (1c, 1p, 1s, 1f, 2c, 2p, 2s, 2f) showed antioxidant activity in the linoleic acid model, and all but 1p showed activity in the DPPH model. The authors concluded that avenanthramides containing caffeic (1c, 2c) and sinapic acid (1s, 2s: 1s and 2s are not natural products) were the most effective antioxidants (Bratt et al., 2003).

The research data on the bioavailability of avenanthramides are limited. In one study, the bioavailability of avenanthramides 2c, 2p, and 2f was determined in six elderly subjects who had consumed an avenanthramide-enriched mixture. Maximum plasma concentrations after consuming 59 and 118 mg of avenanthramides were 167.5 and 559.6 nM, respectively (Chen, Milbury, Collins, & Blumberg, 2007). Recently, Koenig, Dickman, Wise, and Ji (2011) reported that 2c, 2f, and 2p can be detected in the circulating blood of rats following oral intake and are taken up by hepatic, cardiac, and skeletal muscle tissues. Although avenanthramide bioavailability in rats is low compared with that of humans and hamsters, the rank order of plasma concentration is the same: 2p ≫ 2f > 2c. A study investigating the *in vivo* antioxidant activity of avenanthramides in rats demonstrated that 2c attenuates ROS production in some tissues and increases the activity of certain antioxidative enzymes (Ji, Lay, Chung, Fuy, & Peterson, 2003). In addition, avenanthramides enhance antioxidant capacity in humans and act synergistically with vitamin C to protect against LDL-oxidation in hamsters (Chen, Milbury, Kwak, Collins, et al., 2004). In a human study, a total of 120 healthy individuals were randomly assigned to daily supplementation of oat avenanthramides (3.12 mg daily) or a placebo for 1 month (Liu, Yang, Hou, Yao, et al., 2011). In the group receiving avenanthramides, serum levels of superoxide dismutase and reduced glutathione increased by 8.4% and 17.9%, respectively ($p < 0.05$). These results suggest that the antioxidant effect of the avenanthramides is more associated with an indirect antioxidant response via increased activity of endogenous antioxidant enzymes *in vivo* (Dinkova-Kostova et al., 2007).

The anti-inflammatory and antiatherogenic properties of avenanthramides have also been investigated. Avenanthramides are able to suppress vascular endothelial cell expression of adhesion molecules (ICAM-1, VCAM-1, and E-selectin), thereby preventing monocyte adhesion to human aortic endothelial cell monolayers and down-regulating the production of inflammatory cytokines and chemokines (e.g., IL-6, IL-8, and MCP-1) (Liu, Zubik, Collins, Marko, & Meydani, 2004). By up-regulating the p53-p21cip1 pathway and inhibiting the phosphorylation of retinoblastoma protein, 2c inhibits smooth muscle cell proliferation in humans and increases nitric oxide production, two key factors in the prevention of atherosclerosis (Nie, Wise, Peterson, & Meydani, 2006). An avenanthramide enriched oat extract as well as synthetic 2c and its methyl ester decreased proinflammatory cytokine production and adhesion molecule expression in human aortic endothelial cells by inhibiting NF-κB activation (Guo, Wise, Collins, & Meydani, 2008). In keratinocytes, the anti-inflammatory activity of avenanthramides at concentrations as low as 1 ppb prevented the degradation of IκB and decreased phosphorylation of the p65 subunit (Sur, Nigam, Grote, Liebel, & Southall, 2008). Treating cells with avenanthramides inhibited tumor necrosis factor-alpha (TNF-α)-induced NF-κB activity and subsequently reduced interleukin-8 release.

Phenolic compounds exert their antioxidant activity through two main mechanisms: by acting as hydrogen atom donators or as metal ion chelators. The antioxidant capacities of phenolics depends on the number and arrangement of hydroxyl groups, the nature of the substituents in the ring structures, ionization state, steric hindrance, and the stability of the resulting phenoxy radicals. Although the antioxidant activity of oat avenanthramides has been extensively documented, no studies have investigated the antioxidant capacities of 2c, 2f, and 2p in scavenging different types of free radicals, nor has their relative effect in inhibiting the activation of NF-κB been evaluated. The objective of this work was therefore to evaluate the free radical scavenging capacity of synthesized 2c, 2f, and 2p and assess their ability to inhibit TNF-α-induced NF-κB activation *in vitro*.

2. Materials and methods

2.1. Chemicals and apparatus

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), dihydrorhodamine 123 (DHR-123), and disodium fluorescein (3′,6′-dihydroxyspiro[isobenzofuran-1[3*H*],9′[9*H*]-xanthen]-3-one) were purchased from Sigma–Aldrich (St. Louis, MO, USA), and 3-morpholinosydnonimine, hydrochloride (SIN-1) was obtained from Toronto Research Chemicals (North York, ON, Canada). The compound 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA, USA). Hydroethidine fluorescent stain was purchased from Polysciences, Inc. (Warrington, PA, USA). Xanthine, xanthine oxidase from buttermilk, superoxide dismutase from bovine erythrocytes, and