Effects of *Humulus lupulus* extract or its Components on Viability, Lipid Peroxidation, and expression of Vascular Endothelial Growth Factor in Melanoma Cells and Fibroblasts

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Abstract

Cancer and aging are associated with altered cell viability and angiogenesis, which is mediated by vascular endothelial growth factor (VEGF). These alterations have been associated with cellular oxidative stress. *Humulus lupulus* (HOP) extract, and its components, which include alpha-acid, beta-acid, xanthoflavonoids, xanthohumunol, and isoxanthohumunol, exhibit antioxidant activity. This research examined the effects of HOP extract or its components on direct antioxidant activity, and on cell viability, lipid peroxidation, and expression of VEGF in melanoma cells, and dermal fibroblasts. The HOP extract, and its components exhibited direct antioxidant activity. In melanoma cells, HOP extract, and its components significantly inhibited cell viability and stimulated extracellular lipid hydroperoxides at all examined concentrations; and with few exceptions did not significantly alter intracellular lipid peroxidation or the expression of VEGF. In dermal fibroblasts, HOP extract and its components significantly inhibited cell viability and intracellular lipid peroxidation, and stimulated the expression at VEGF at the highest examined concentration; and with few exceptions did not significantly alter extracellular lipid peroxidation. It is inferred that HOP extract, and its components differentially and beneficially regulate the cellular biochemistry of melanoma cells, and fibroblasts for the management of cancer, and aging.

Keywords: *Humulus lupulus*; Melanoma; Fibroblasts; Cell Viability; Lipid Peroxidation; Vascular Endothelial Growth Factor.

Introduction

Carcinogenesis and aging are associated with alterations in cell viability, cell migration, angiogenesis, and extracellular matrix (ECM), which is predominantly synthesized by dermal fibroblasts [1-8]. While carcinogenesis and aging involve increased ECM proteolysis, cancer is associated with increased cell survival and angiogenesis, and aging is associated with reduced cell viability and angiogenesis, which is mediated by several factors including vascular endothelial growth factor (VEGF) [1-8]. Oxidative stress plays a key role in aging and carcinogenesis [1-5]. The reactive oxygen species (ROS) cause oxidative damage to DNA, proteins and lipids, which includes lipid peroxidation [2-4].
Materials and Methods

Antioxidant Activity

The direct antioxidant activity of HOP extract or its components was determined by ABTS® (2, 2′-azino-di-[3-ethylbenzthiazoline sulphonate]) assay, based on the inhibition of the oxidation of ABTS® to ABTS® radical by metmyoglobin or by antioxidants (Cayman Chemical Antioxidant Assay kit) [1]. HOP extract or its components (alpha-acid, beta-acid, xanthoflavonoids, xanthohumulonol and isoxanthohumulonol) at 0, 0.01, 0.04, 0.02 or 1% of respective 50mg/ml stock solutions, was incubated with ABTS and metmyoglobin and the inhibition of ABTS oxidation was measured spectrophotometrically at 405 nm. The HOP extract and its components were obtained from S. S. Steiner, Inc.
Results

Direct inhibition of ABTS oxidation by HOP extract, and its components

The HOP extract, and its components exhibited significant direct antioxidant activity (p<0.05) (Figure 1). Relative to control, 1% HOP extract, alpha-acid, beta-acid, xantho-flavonoid, xanthohumol, and isoxanthohumol significantly inhibited ABTS oxidation to 75%, 32%, 51%, 66%, 60% and 91% of control, respectively (p<0.05) (Figure 1). In addition, 0.2% of HOP extract, alpha-acid, xanthohumol, and isoxanthohumol significantly inhibited ABTS oxidation to 46%, 37%, 86%, and 70% of control, respectively (p<0.05) (Figure 1).

Regulation of melanoma and fibroblast cell viability by HOP extract, and its components

The HOP extract or its components significantly inhibited melanoma cell viability at each of the concentrations, 0.01 to 0.2%; and fibroblast cell viability predominantly at the highest concentration, 0.2% (p<0.05) (Figure 2).

Regulation of melanoma and fibroblast extracellular hydroperoxides by HOP extract, and its components

The HOP extract or its components significantly stimulated extracellular lipid hydroperoxides in melanoma cells at each of the concentrations, 0.01 to 0.2% (except xanthohumol at 0.01%); and in fibroblast cells at the highest concentration (except xantho-flavonoid, xanthohumol, and isoxanthohumol), 0.2% (p<0.05) (Figure 3).
In fibroblasts, 0.2% HOP extract, alpha-acid, and beta-acid significantly stimulated extracellular hydroperoxides to 122%, 130%, and 169% of control, respectively (p<0.05) (Figure 3B).

Regulation of melanoma and fibroblast intracellular hydroperoxides by HOP extract, and its components

The HOP extract or its components did not significantly alter intracellular lipid hydroperoxides in melanoma cells (except xantho-flavonoid and xanthohumol at 0.2%); and significantly inhibited it in fibroblast cells at the highest concentration, and in addition the second highest concentration for xantho-flavonoid, and xanthohumol (p<0.05) (Figure 4).

In melanoma cells, 0.2% xantho-flavonoid and xanthohumol significantly stimulated intracellular hydroperoxides to 216% and 220% of control, respectively (p<0.05) (Figure 4A).

In fibroblasts, 0.2% HOP extract, alpha-acid, beta-acid, xantho-flavonoid, xanthohumol, and isoxanthohumol significantly inhibited intracellular lipid peroxidation of melanoma cells (A), and fibroblast cells (B); error bars: standard deviation, n=4; *: p<0.05, relative to control.

In melanoma cells, 0.04 and 0.2% of xantho-flavonoid significantly inhibited VEGF expression to 60% and 53% of control, respectively (p<0.05) (Figure 5A).

In fibroblasts, 1% HOP extract, alpha-acid, beta-acid, xantho-flavonoid, xanthohumol, and isoxanthohumol significantly stimulated VEGF expression to 112%, 121%, 121%, 124%, 149%, and 132% of control, respectively (p<0.05) (Figure 5B). In addition, 0.04% iso-xanthohumol significantly stimulated VEGF expression to 146% of control, respectively (p<0.05) (Figure 5B).

Discussion

Carcinogenesis and angiogenesis are associated with altered cellular redox balance due to exposure to environmental pollutants or ultraviolet radiation, and genetics [1-5]. Phenolic compounds with their antioxidant property have potential to modulate the cellular redox environment and thereby carcinogenesis or aging [1-5]. The differential beneficial effects of HOP extract or its extracts in cancer versus normal cells are from their pro-oxidant versus antioxidant effects, respectively, in these cells. The potential for differential effects, in cancer versus normal cells, may be from differential cellular environment in cancer versus normal cells, such as the concentrations of metals that catalyze Fenton or Haber-Weiss reactions [10, 11]. While the HOP extract, and its components exhibited direct antioxidant activity, they had differential beneficial effects in melanoma cells, and dermal fibroblasts.

Carcinogenesis is associated with increased cell proliferation, and angiogenesis [1-5]. The HOP extract or its components, from the smallest to the highest concentration examined, significantly inhibited melanoma cell viability and
stimulated extracellular hydroperoxides, suggesting pro-oxidant mechanism in the inhibition of melanoma cell viability. In addition, xantho-flavonoid significantly inhibited the expression of VEGF in melanoma cells, suggesting anti-angiogenic potential.

Aging is associated with loss of cell viability, and reduced angiogenesis [1-5]. The HOP extract or its components, at the highest concentration examined, significantly inhibited fibroblast cell viability and stimulated extracellular hydroperoxides, suggesting pro-oxidant mechanism. However, the HOP extract or its components significantly inhibited intracellular lipid peroxidation in fibroblast cells, suggesting cellular antioxidant activity; and significantly stimulated the expression of VEGF, suggesting angiogenic potential.

Conclusion
Carcinogenesis is associated with increased cell proliferation and angiogenesis, whereas aging is associated with reduced cell viability and angiogenesis. The melanoma cell viability was inhibited through pro-oxidant mechanism, at all concentrations of the HOP extract or its components; without antioxidant cellular effect or increased expression of VEGF. The fibroblasts cell viability was inhibited by pro-oxidant mechanism, at the highest concentration of HOP extract or its components, and was associated with increased cellular antioxidant activity and expression of VEGF.

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