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Potential Cell Culture Models for Antioxidant Research

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The antioxidant activity of pure compounds, foods, and dietary supplements has been extensively studied with the development of many new antioxidant and antioxidant activity assays in recent years. However, these assays, such as total phenolics, total flavonoids, and total antioxidant activity in vitro, do not reflect the cellular physiological conditions and do not consider the bioavailability and metabolism issues. In addition, the mechanisms of action of antioxidants go beyond the antioxidant activity scavenging free radicals in disease prevention and health promotion. Animal models and human studies are expensive and not suitable for initial antioxidant screening of foods and dietary supplements. Therefore, there is a need for cell culture models to access the bioactivity of antioxidants. This paper is an overview of cell culture models for antioxidant research, as reported at the First International Congress on Antioxidant Methods, held in Orlando, FL, June 16−18, 2004, and outlines potential cell culture models for initial antioxidant screening and antioxidant research.

KEYWORDS: Antioxidants; foods; antioxidant activity; cancer; cardiovascular disease; cell culture models

INTRODUCTION

Free radical induced oxidative stress has been hypothesized to be a major factor in the development of several degenerative chronic diseases. Oxidative stress can cause oxidative damage to large biomolecules such as lipids, proteins, and DNA, resulting in an increased risk for inflammatory diseases, cardiovascular disease (CVD), cancer, diabetes, Alzheimer's disease, cataracts, and age-related functional decline (*1*−*3*). To prevent or slow the oxidative stress induced by free radicals, sufficient amounts of antioxidants need to be consumed. Fruits, vegetables, and whole grains contain a wide variety of antioxidant compounds (phytochemicals), such as phenolics, flavonoids, and carotenoids (*4*−*9*), and may help to protect cellular systems from oxidative damage and also lower the risk of chronic diseases. The benefits of fruits and vegetables have been consistently supported by epidemiological studies reporting that the regular consumption of fruits and vegetables as well as whole grains is associated with a reduced risk of developing chronic diseases such as cancer and CVD (*10*−*13*). Bioactive nonnutrient phytochemicals in fruits, vegetables, whole grains, and other plant foods have been linked to the reduced risk for major chronic diseases, including cancer and CVD (*14*).

Antioxidant research has been expanded dramatically since the mid-1990s with the development of several assays measuring the total antioxidant activity of pure compounds, foods, and dietary supplements (*15*−*18*). However, these total antioxidant activity assays in test tubes do not necessarily reflect the cellular physiological conditions and do not consider the bioavailability and metabolism issues. In addition, the mechanisms of action of antioxidants go beyond the antioxidant activity of scavenging free radicals in disease prevention and health promotion (*19*). Animal models and human studies are expensive and not suitable for the initial antioxidant screening of foods and dietary supplements. Therefore, there is a need for cell culture models to support antioxidant research prior to animal studies and human clinical trials. This paper gives an overview of cell culture models for antioxidant research, as reported at the First International Congress on Antioxidant Methods, held in Orlando, FL, June 16−18, 2004, and outlines potential cell culture models for initial antioxidant screening and antioxidant research.

POTENTIAL CELL CULTURE MODELS FOR CANCER RESEARCH

Cells in humans and other organisms are constantly exposed to a variety of oxidizing agents, some of which are necessary for life. These agents may be present in air, food, and water, or they may be produced by metabolic activity within cells. The key factor is to maintain a balance between oxidants and antioxidants to sustain optimal physiological conditions. Overproduction of oxidants can cause an imbalance leading to oxidative stress, especially in chronic bacterial, viral, and parasitic infections (*2*). Oxidative stress can cause oxidative damage to large biomolecules such as lipids, proteins, and DNA, resulting in an increased risk for cancer.

Carcinogenesis is a multistep process, and oxidative damage is linked to the formation of tumors through several mechanisms (*2*, *3*). Oxidative stress induced by free radicals can cause DNA damage, which, when left unrepaired, can lead to base mutation, single- and double-strand breaks, DNA cross-linking, and

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Table 1. Proposed Mechanisms of Action by Which Dietary Antioxidants May Prevent Cancer

antioxidant activity
scavenge free radicals and reduce oxidative stress
inhibition of cell proliferation
induction of cell differentiation
inhibition of oncogene expression
induction of tumor suppress gene expression
induction of cell cycle arrest
induction of apoptosis
inhibition of signal transduction pathways
enzyme induction and enhancing detoxification
phase II enzyme
glutathione peroxidase (GPX)
catalase
superoxide dismutase (SOD)
enzyme inhibition
phase I enzyme (block activation of carcinogens)
cyclooxygenase-2 (COX-2)
inducible nitric oxide synthase (iNOS)
xanthine oxide
enhancement of immune functions and surveillance
anti-angiogenesis
inhibition of cell adhesion and invasion
inhibition of nitrosation and nitration
prevention of DNA binding
regulation of steroid hormone metabolism
regulation of estrogen metabolism
antibacterial and antiviral effects

Table 2. Proposed Mechanisms of Action by Which Dietary Antioxidants May Prevent CVD

antioxidant activity
scavenge free radicals and reduce oxidative stress
prevent LDL oxidation
induction of expression of hepatic LDL receptors
regulation of sterol regulatory element binding proteins (SREBPs)
modulation of cholesterol synthesis
regulation lipid profiles
inhibition of cholesterol absorption
regulation of prostanoid synthesis (PGE_2)
reduction of platelet aggregation
regulation of nitric oxide ($NO^{\bullet}$) production
lowering C-reaction protein
regulation of blood pressure

chromosomal breakage and rearrangement (*3*). This potentially cancer-inducing oxidative damage might be prevented or limited by dietary antioxidants found in fruits, vegetables, and other plant foods. Studies to date have demonstrated that the mechanisms of action of antioxidants in the prevention of cancer go beyond the antioxidant activity of scavenging free radicals. Antioxidants in fruits, vegetables, whole grains, and other plant foods can have complementary and overlapping mechanisms of action (**Table 1**), including the following: antioxidant activity and scavenging of free radicals; regulation of gene expression in cell proliferation, cell differentiation, oncogenes, and tumor suppressor genes; induction of cell cycle arrest and apoptosis; modulation of enzyme activities in detoxification, oxidation, and reduction; stimulation of the immune system; regulation of hormone-dependent carcinogenesis; inhibition of arachidonic acid metabolism; and antibacterial and antiviral effects (*4*, *5*, *19*−*22*). Therefore, the potential cell culture models for cancer research should include this line of research (**Table 3**). Obviously, no one cell culture system does it all.

Table 3. Potential Cell Culture Models for Antioxidant Screening

cell culture model	biomarker
cancer	
antiproliferation	inhibition of proliferation
Caco-2 colon cancer cells	
HepG2 liver cancer cells	
MCF-7 breast cancer cells	
cell cycle arrest	G1 arrest, G1/S ratio
apoptosis	induction/inhibition of apoptosis
antiangiogenesis	inhibition of angiogenesis, MMP2
COX-2 inhibition	COX-2 expression, PGE_2
quinone reductase	induced quinone reductase activity
oxidative DNA damage	8-OH-dG
CVD	
inhibition of cholesterol synthesis	cholesterol, SREBPs
expression of hepatic LDL receptors	LDL receptors, cellular LDL uptake
bioavailability of antioxidant	
flavonoid bioavailability	cellular flavonoid uptake
carotenoid bioavailability	cellular carotenoid uptake
metabolism of antioxidant	
primary haptocytes	metabolic compound(s)
Caco-2 colon cancer cells	metabolic compound(s)
HepG2 liver cancer cells	metabolic compound(s)

POTENTIAL CELL CULTURE MODELS FOR CVD RESEARCH

Several mechanisms for the prevention of atherosclerosis by dietary antioxidants in fruits and vegetables have been proposed (**Table 2**). In the low-density lipoprotein (LDL) oxidation hypothesis, oxidized LDL cholesterol has been suggested as the atherogenic factor that contributes to CVD (*23*, *24*). When circulating LDLs are present at high levels, they infiltrate the artery wall and increase intimal LDL, which can then be oxidized by free radicals. This oxidized LDL in the intima is more atherogenic than native LDL and serves as a chemotactic factor in the recruitment of circulating monocytes and macrophages. Oxidized LDL is typically taken up by macrophage scavenger receptors, thus inducing the formation of inflammatory cytokines and promoting cell proliferation, cholesterol ester accumulation, and foam cell formation. Gruel-like, lipid-laden foam cell accumulation in the blood vessel, forming fatty streak, would cause further endothelial injury and lead to atherosclerotic disease. Because oxidized LDL plays a key role in the initiation and progression of atherosclerosis, giving dietary supplements of antioxidants capable of preventing LDL oxidation has been an important therapeutic approach. Dietary antioxidants that are incorporated into LDL are themselves oxidized when the LDL is exposed to pro-oxidative conditions; this occurs before any extensive oxidation of the sterol or polyunsaturated fatty acids can occur (*25*). Therefore, dietary antioxidants might retard the progression of atherosclerotic lesions. In addition, phytochemicals have been shown to have roles in the reduction of platelet aggregation, modulation of cholesterol synthesis and absorption, and reduction of blood pressure. Recently, C-reactive protein, a marker of systemic inflammation, has been reported to be a stronger predictor of CVD than is LDL cholesterol (*26*), suggesting that inflammation is a critical factor in CVD. Inflammation not only promotes initiation and progression of atherosclerosis but also causes acute thrombotic complications of atherosclerosis (*27*). Dietary phytochemicals can lower C-reactive protein dramatically. Therefore, the anti-inflammatory activity of phytochemicals may play an important role in the prevention of CVD. Dietary antioxidants also have complementary and overlapping mechanisms of action in the prevention of CVD (**Table 2**).

BIOAVAILABILITY AND METABOLISM OF ANTIOXIDANTS

Bioavailability and metabolism are two important questions that need to be addressed in the study of the biological effects of antioxidants (phytochemicals) in foods. The form of antioxidants found in foods is not necessarily the same as the form found in the blood or the targeted tissues after digestion, absorption, and metabolism. To study the mechanisms of action of antioxidants in the prevention of chronic disease, two important questions to be asked are the following:

Are these antioxidants (phytochemicals) bioavailable? Are these original antioxidants or their metabolites the bioactive compounds? It is crucial to understand the bioavailability and metabolism of these compounds to gain knowledge of what compounds and how much of these compounds actually reach target tissues. In some cases, the original phytochemicals may be excreted or metabolized and never actually reach target tissue, and the active compounds may not be the original antioxidant compounds found in foods but rather their metabolites. To this date, many studies did not address the bioavailability and metabolism of phytochemicals from whole foods.

Examining the bioavailability of compounds from food sources can be challenging, because there are many factors that may influence bioavailability. Foods contain a wide variety of phytochemicals, and interactions with other chemicals in the food may affect bioavailability. Phytochemicals may be bound to different sugars (glucosides, xylosides, rhamnosides, galactosides) or to other compounds (fibers) that may affect the compound's bioavailability. Other factors, such as digestion, food processing, and stage of harvest, may also affect phytochemical bioavailability. Although much progress has been made in understanding the bioavailability and further metabolism of pure compounds, more work is needed to further comprehend the bioavailability of phytochemicals from complex food sources.

A good in vitro model would be beneficial in this area of study in evaluating the bioavailability of phytochemicals from foods by offering a simple method to screen for factors that may affect intestinal absorption of phytochemicals, such as the food matrix, food processing, digestion, and interactions with other foods. Human and animal models can be expensive and time-consuming, whereas a cell culture model allows for rapid, inexpensive screenings. The Caco-2 cell culture model has the potential to be a good model to measure the bioavailability of antioxidants, such as carotenoids and flavonids, from whole foods (*28*−*32*).

CONCLUSIONS

On the basis of the proposed mechanisms of action by which dietary antioxidants prevent cancer and CVD (**Tables 1** and **2**), a number of potential cell assays are suggested for initial antioxidant screening and antioxidant research (**Table 3**). Future research is needed to develop cell-based antioxidant activity assays with considerations of bioavailability and metabolism of antioxidants. Mechanism-based cell culture models are valuable in future antioxidant research.

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