CHEMISTRY AND BIOLOGICAL ACTIVITIES OF GRAPES

John M. Pezzuto and **Tamara P. Kondratyuk**
Arnold & Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, 75 DeKalb Avenue, Brooklyn, New York 11201

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Summary

Grapes and grape-derived products have been incorporated into the diet and lifestyle of humans throughout recorded history. A spike in scientific interest was generated in 1992 when the cardioprotective effect of red wine was postulated. Greatest attention focused on high content of polyphenolic antioxidants, the capacity of which may exceed that of vitamins C and E. We describe recent advancements in grapes, grape phytochemicals, and associated molecular mechanisms. We illustrate polyphenols as a functional food, their role in cancer chemoprevention, heart disease, aging, and as important components of wine.

Grapes and grape products are a key dietary source of resveratrol. There were few studies prior to 1997 when we first described the potential of resveratrol to modulate various targets associated with multiple stages of carcinogenesis. Subsequently, more than 15,000 reports have appeared in the scientific literature. Here we describe the chemopreventive and antioxidant activity of resveratrol, its relation to different stages of carcinogenesis, and pleiotropic effects. We present data related to metabolites and recent work to generate chemical derivatives with greater specificity and potency.

Given the historical significance of wine, we describe some chemical and biological characteristics. The benefits of moderate wine consumption appear to be greater than those of other alcoholic beverages. Some evidence indicates wine polyphenols could reduce atherosclerosis, lower blood pressure, inhibit platelet aggregation, reduce inflammation, and mediate a cancer chemoprevention response.

The goal of this chapter is to illustrate the biological potential of grapes, as well as their chemical complexity and diversity. We discuss the challenges associated with attempting to pinpoint the mechanism(s) of action of grapes. Overall, it seems reasonable to conclude dietary consumption of grapes and/or grape-related products, along with maintaining an active healthy lifestyle, are beneficial for the general population.

1. Introduction

The recognition of the healing power of plants is an ancient phenomenon. Traditional healers used plants to prevent or cure various disease states. Hippocrates (460-377 B.C.) is frequently quoted as recommending, “Let thy food be thy medicine and thy medicine be thy food.” In support of this supposition, the incidence of cancer and cardiovascular diseases is relatively low in Asian countries such as India and China, where vegetables, fruits and spices are a major part of the human diet. Botanicals have been used by human beings throughout history, and current-day medical literature documents the widespread use of herbal remedies in many countries throughout the world. Considerable epidemiological and experimental evidence has been accumulated indicating risk reduction for numerous cancers. In addition, the presence of certain phytochemical constituents supports the pharmacological and physiological efficacy of some botanical treatments. Overall, it seems reasonable to promulgate the concept of dietary components contributing to health and well-being.

Grapes have been described in numerous biblical stories, being referred to as “fruit of the vine.” Grapes were cultivated in Asia as early as 5000 BC, but grow wild in many continents (Europe, Asia, Africa, and North America). European travelers brought ("old World") grapes to the United States. Now in the United States, over 90% of grapes come from California.

Although the potential of grapes to mediate various biological effects remains under active investigation, health benefits have been recognized for many centuries. In ancient Egypt, sap from grapevines was used in ointments to treat skin diseases. The fruit was crushed into wine elixirs or ripened to serve as therapeutics for nausea, constipation, cholera, smallpox, liver disease, and cancer. Ancient Egyptian wine was made from a domesticated grape, and is believed to have been fermented by natural yeasts from the grape skin. Some wines were sweetened with figs and raisins, and flavored with herbs, having medical benefits. Grape leaves were used as a primary treatment for heart disease and infection.

In the past century, disease states such as hypertension, coronary heart disease, and stroke have become markedly more prevalent. Despite active management of cardiovascular disease, the societal burden remains high. Lifestyle and dietary approaches attract increasing interest in the etiology of heart disease over the past few decades. The cardioprotective benefits of diets rich in grapes have been studied most extensively, but various other activities remain under active investigation. For example, during the first Grape Health Workshop sponsored by NGWI (National Grape and Wine Initiatives, a nationwide coalition representing all segments of the grape industry), several key areas of interest for further development were discussed, including

cardiovascular disease, diabetes/glycemic response, immune function, cancer, cognitive function, antimicrobial/antiadhesion, and bioavailability/analytical strategy. Certainly, however, the most systematic and comprehensive examination of the relationship between grapes and human health has been supported and administered by the California Table Grape Commission. Over 60 studies have been or are being conducted to investigate the biological potential of a standardized grape powder that is representative of a generic grape (e.g., red, green, purple). Some of this work has been reviewed in the literature.

The grape received some attention when epidemiological studies linked moderate consumption of red wine with increased longevity and reduction of the incidence of cardiovascular diseases, a phenomenon known as the 'French paradox'. Our interest has been in the area of cancer chemoprevention, a strategy for preventing or delaying human malignancies. Natural product chemopreventive agents, many of which are found in the diet of human beings, have received great attention. In 1997, we demonstrated the ability of resveratrol (3,4′,5-*trans*-trihydroxystilbene) to inhibit multiple stages of carcinogenesis. The primary dietary source of resveratrol is grapes and wine, although it is found in a few other sources, such as mulberries and peanuts. Our work led to a rapid intensification of research activity involving resveratrol. As of November 2015, our seminal work with resveratrol has been cited over 3,100 times, and nearly 24,000 manuscripts concerning resveratrol have been published in the scientific literature since 1997. Nonetheless, the molecule is unusually promiscuous and specific mechanisms remain elusive. Considering the structural simplicity of this stilbene, the intensity of interest is phenomenal.

2. Health Promoting Grape Nutrients

There are many examples of grapes being utilized for the promotion of good health. Grape seeds, skin, leaves, stems are used in herbal remedies, and a diet consisting completely of grapes has been described as a successful treatment for cancer. Fresh, preserved, and dried grapes are used in a diet, or in the form of liquid extracts, tinctures, and gargles in medicine. Grape seed extract and some other preparations including resveratrol are available as dietary supplements. Grapes are an excellent source of vitamins A, C, B6 and folate. In addition, they contain potassium, calcium, magnesium, iron, selenium, phosphorus, zinc, manganese and copper, as well as proteins and fibers. Some scientific evidence suggests certain chemicals in grapes may help prevent heart disease and cancer, but of course it is difficult to determine the role of one particular food in disease prevention. A balanced diet that includes several servings a day of fruits and vegetables, along with nuts, seeds, whole grain cereals, and beans, is likely to be more effective than eating one food in a large amount.

As mentioned above, resveratrol is one unique component of grapes that has received a great deal of attention. However, a broader phytochemical analysis demonstrates a vast array of constituents, many of which are capable of mediating biological responses. For example, a search of the NAPRALERT database (the world's first computerized natural product alert database) revealed over 270 citations reporting over 1600 compounds representing over 30 chemical classes (Table 1). Consequently, when considering biological responses that could be mediated by grapes, it would seem necessary to take

into account each of these constituents, their metabolites, all of the mechanisms and pathways modulated by these constituents and their respective metabolites, and how all of these factors work in harmony.

Alicyclic	Carbohydrate	Oxygen heterocycle
Alkaloid	Coumarin	Phenol
Alkane	Flavone	Phenylpropanoid
Alkanol	Flavonol	Proteid
Alkanone	Flavonoid	Quinoid
Alkenal C5 or more	Indole alkaloid	Sesquiterpene
Anthraquinone	Inorganic substance	Steroid
Benzenoid	Isoquinoline alkaloid	Stilbene
Bibenzyl	Lignan	Tannin
Carotene	Lipid	Triterpene
Carotenoid	Monoterpene	

[1]A total of 1620 compounds were reported within these chemical classes.

Table 1. Chemical classes reported in *V. vinifera* and *V. saccharifera*[1]

Obviously, this is a daunting task, but only the tip of the iceberg. In order to realistically assess how diet affects human health and well being, all components should be taken into account. Many fruits and vegetables have been examined, and a myriad of chemical compounds have been identified. So, as is the case with the scores of compounds associated with the grape, it makes sense to consider the biologic potential of each of these molecules as well as their respective metabolites. In addition, all of these compounds may be affected by food processing, intestinal microflora, selective tissue distribution, etc. Thus, the overall influence of diet on human beings is not a straightforward matter. Nonetheless, using a much less holistic approach, numerous studies have been performed with grapes or grape components, and some of this information will be presented herein.

Ischemic heart disease is the leading causes of death within the group of cardiovascular diseases (CVD). CVD is one of the leading causes of death in Western countries. A popular herbal supplement among patients suffering from cardiovascular disease is grape seed extract (GSE). Herbal extracts, being composed of a mixture of active compounds, could act through more than one mechanism, and hence could potentially exert multiple effects. Emphasis has been placed on antioxidant activity, since GSE is effective in this capacity. Antioxidants are compounds that inhibit the action of free radicals, such as activated oxygen molecules, that can damage cells. It has also been proposed that herbal flavonoids in GSE may play a vital role in modulating cardiomyocyte response against reperfusion injury. Further exploration of pathophysiological mechanisms of GSE in modulating cardiovascular function should help to define the actual role of botanical flavonoids in the treatment and prevention of coronary heart disease.

In addition to cardiovascular diseases, antioxidants are considered useful for treating many medical conditions, such as arthritis, allergies, circulatory problems, diabetes, and some types of cancer. Epidemiological studies suggest that lifestyle, geographical

location, ethnicity, sex, age, and trans-migratory populations, are major influencing factors in the incidence of cancer. Considerable scientific interest has been generated in developing various preventive measures based on diet, especially those involving fruits and vegetables. A cancer chemopreventive agent could be effective at any of the stages of carcinogenesis: initiation, promotion, and progression. GSE is a nutraceutical agent that is commonly consumed as a health/dietary supplement and is sold as an over-the-counter product in the form of capsules or tablets (100–500 mg). Consumer interest in GSE has been primarily due to the high content of antioxidants in the form of proanthocyanidins in this extract. Antioxidant capacity of GSE is greater than that of known antioxidants such as vitamins C and E. As a cancer chemopreventive, agent GSE demonstrates promising results in the mouse skin carcinogenesis model. Topical application of GSP (polyphenolic fraction of GSE) to 7,12-dimethylbenz(*a*)anthracene (DMBA)-initiated dorsal mouse skin resulted in a highly significant inhibition of 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-promoted skin cancer, as evidenced by a significant reduction in tumor incidence, multiplicity, and volume.

In addition to skin, inhibition of other tumor types has been demonstrated by treatment with GSE that halts the growth of human colorectal carcinoma cells in culture and, more importantly, inhibits the growth of these cells as tumor xenografts in athymic nude mice. Similarly, it has been shown that GSE induced apoptotic death in DU145 human prostate cancer cells, and oral feeding of GSE to TRAMP (transgenic adenocarcinoma of the mouse prostate) mice resulted in a decrease of aberrant cell cycle progression.

More specifically for the treatment or prevention of breast cancer, one therapeutic approach is to target the cytochrome P450 enzyme aromatase. Procyanidin dimers, found in high quantities in grape seeds, inhibit the activity and expression of this enzyme. In related work, proanthocyanidins were found to inhibit the biosynthesis of estrogen, which could possibly affect hormone-sensitive tumors, including some breast tumors. In another study, GSE exerted a synergistic effect with doxorubicin in inhibiting the growth of estrogen-receptor–expressing MCF7 (ATCC® HTB-22™) cells as well as estrogen-receptor negative MDA-MB-468 (ATCC® HTB-132™) cells. Clinical trials in Mayo Clinic Clinical Trials.gov Identifier NCT00566553 are currently in progress to study the potential of GSE to suppress estrogen levels for breast cancer prevention.

3. Polyphenols

As a plant grows in the wild or under cultivation, different polyphenols can be generated as a defensive response against stress from ultraviolet radiation, pathogens, and physical damage. More than 8000 phenolic structures are known, including 4000 flavonoids. The diversity of polyphenols in plants has led to different classification methods according to their source of origin, biological function, and chemical structure. Polyphenols are divided into groups depending on the number of phenol rings and groups attached to the rings. Three phenolic compounds in Figure 1 are derivatives of hydroxybenzoic acid (vanillic, gallic) and hydroxycinnamic acid (coumaric). The most common polyphenols are phenolic acids, simple molecules that include the widely distributed hydroxybenzoic and hydroxycinnamic acids (Figure 1). Also, the majority of polyphenols in plants exist as glycosides with sugar units at various positions of the basic structural skeletons.

Flavonoids make up the largest collection of polyphenols. Flavonoids can be divided into five groups based on the junction of the phenol rings and the level of polymerization: major flavonoids, isoflavonoids, neoflavonoids, tannins and minor flavonoids. Major flavonoids can be divided into several subclasses such as flavones, flavanones, flavonols, flavanols, flavanonols, anthocyanidins, and biflavonoids. They are widely distributed in nature and a prevalent component in the human diet. Great emphasis has been placed on the study of methoxylated flavonoids, which may demonstrate more favorable pharmacokinetic properties.

A number of excellent reviews dealing with their structure, absorption, metabolism, and pharmacokinetics have been published, including our recent review highlighting flavonoids initiating responses that may be relevant to cancer chemoprevention, antioxidation and anti-inflammation. The capacity of flavonoids to act as an antioxidant is dependent on their molecular structure and the position of hydroxyl groups and other substitutions. In addition, the prooxidant capacity of flavonoids may be relevant for the treatment of cancer. Some clinical trials or meta-analyses have suggested positive associations between flavonoid intake and human health, whereas others have not supported such a relationship.

In addition to the phenolic acids and Flavonoids, there are several non-flavonoid polyphenols found in foods that are important to human health. Among these, resveratrol is unique to the grapes and red wine; ellagic acid and its derivatives are found in strawberries, raspberries, and in the skins of different nuts. Lignans exist in the bound forms in flax, sesame and many grains. Curcumin is from turmeric. Rosmarinic acid is a dimer of caffeic acid, and ellagic acid is a dimer of gallic acid.

Plant polyphenols are important part of the human diet, the total intake is approximately 1g/day. The main sources of polyphenols are fruits, vegetables, chocolate, dry legumes, tea, coffee, wine and beer. Polyphenols have received tremendous attention due to their roles in the prevention of degenerative diseases, particularly cancers, cardiovascular diseases and neurodegenerative diseases. They are antioxidants that help reduce oxidative stress caused by excess reactive oxygen species (ROS).

Grapes are a major source of phenolic compounds. The most widespread representative of phenolic acids in grapes and wine are caffeic, vanillic, ferulic and gallic acids (Figure 1). Differences in flavor and color are associated with the content and profile of polyphenols. The concentration of phenolic compounds depends on the vine variety and it is influenced by environment factors as well. Grapes skin phenolics vary widely, distributed among grape cultivars with different genetic backgrounds and originations. High amount of monoglucoside and diglucoside anthocyanins were detected in *V. vinifera* and *V. rotundifolia* grapes, respectively, while both existed in other species and hybrids. 3',4',5'-Substituted and methylated anthocyanins were dominant in grapes originating from Eurasia, while the proportions of total anthocyanins were relatively low in grapes originating from North America. Acetyl anthocyanins were more abundant in *V. vinifera* grapes, quercetin-3-glucuronide and quercetin- 3-glucoside were common in the skins of *Euvitis* species, but were not detected in muscadine grapes. Muscadine grape skin possessed high content of flavonols, flavan-3-ol monomers and ellagic acids. Flavan-3-ols were generally sparse in the skins of the East Asian grapes.

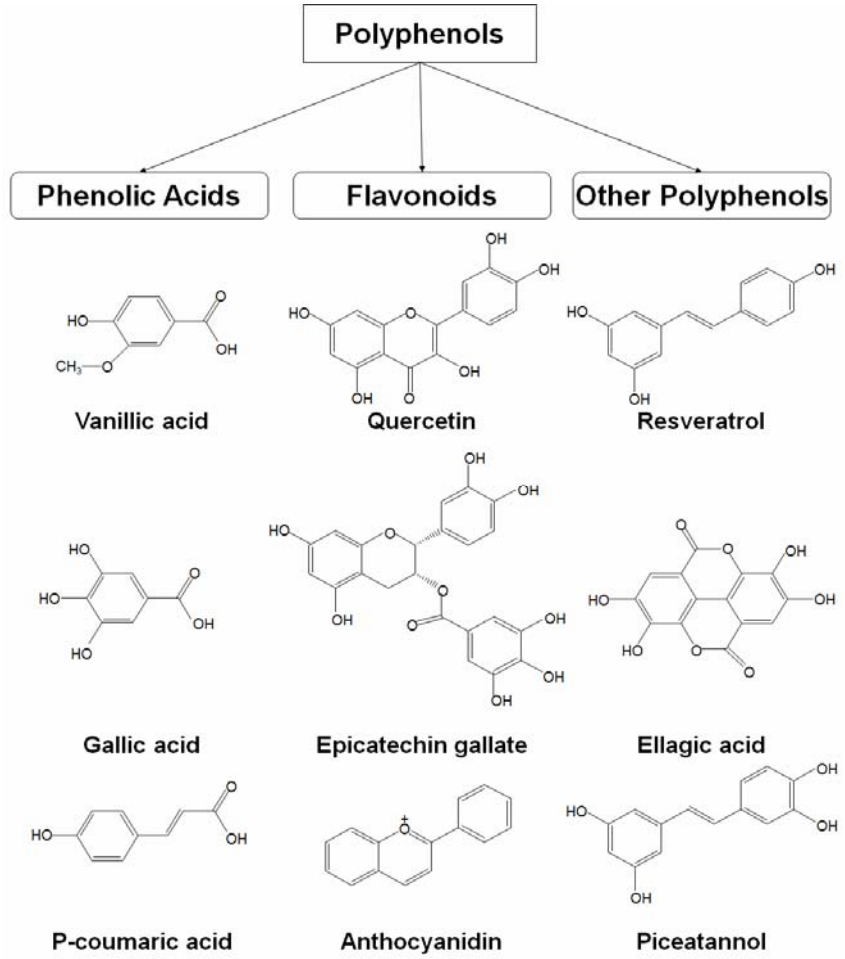

Figure 1. Major polyphenol classes and representative members in grape and wine.

3.1. Multiple Biological Functions Of Dietary Polyphenols

The biological properties of polyphenols depend on a number of factors such as their molecular structure and bioavailability. In some cases, pure compounds have been studied and, in other cases, mixtures or whole foods were investigated. Since some diseases are multifactorial and many normal cellular pathways become aberrant, it is unlikely one agent could be effective against such disorders. In this regard, foods may have the advantage of simultaneously influencing various damaged pathways. *In vitro* and *in vivo* investigations are beginning to shed some light on the link between the dietary intake of polyphenols and health benefits.

3.2. Antioxidant Activities of Dietary Polyphenols

The levels of reactive oxygen species (ROS) can increase rapidly under conditions of oxidative stress and thereby produce a threat to cellular structures and function. This may lead to chronic inflammation and, in some cases, conditions such as cancer and cardiovascular disease. Polyphenols have the ability to interact with ROS and thereby function as antioxidants. In terms of antioxidant activity, some polyphenols are considered even more important contributors than vitamin C. Catechins are primarily

found in grape seeds and have the monomeric flavan-3-ols catechin, epicatechin, gallocatechin, epigallocatechin, epicatechingallate and epigallocatechin-3-gallate. The stilbene resveratrol is found in the skin of grapes. Proanthocyanidins are found in grape seeds and red wine and share common properties with other polyphenols such as an ability to chelate metal ions. Ellagitannins are large molecules and are found in red wine, tea, nuts and pomegranate.

Polyphenols can neutralize free radicals by donating an electron or hydrogen atom. Polyphenols suppress the generation of free radicals, but more frequently, they act as direct radical scavengers of lipid peroxidation chain reactions (chain breakers). In addition to radical scavenging, polyphenols are also known as metal chelators. Chelation of transition metals such as Fe^{2+} and Cu^{2+} can directly prevent oxidation caused by highly reactive hydroxyl radicals. In fact, polyphenols play a vital role (antioxidant effect) in protection of copper-induced DNA damage. Polyphenols can actually function as a co-antioxidant, and are involved in the regeneration of essential vitamins.

Although polyphenols may reduce oxidative stress by the mechanisms described above, the full action of these compounds is more complicated. For example, as antioxidant polyphenols donate an electron or hydrogen atom, they become themselves free radicals that can potentially promote pro-oxidant activities. In addition, experimental data indicates polyphenols and their *in vivo* metabolites may not act as conventional hydrogen- or electron-donating antioxidants, but may regulate protein kinases and lipid kinase signaling pathways in cells. Another complicating factor is that phenolics in plants are glycosylated, and this has an influence on efficiency and absorption. In other cases, concentrations of polyphenols in plasma are too low to exhibit any significant antioxidant activities, and metabolism may be rapid. Only a small fraction of consumed polyphenols is excreted via the urine in humans.

Phytosome technology is one approach to circumvent some of these issues. Conversion of polyphenols to phytosome forms, creating intermolecular bonding between individual polyphenol molecules and one or more molecules of the phospholipids, improves oral bioavailability. As an example, grape seed extract is a concentrated source of polyphenols (GSP), resembling the catechins of green tea in basic molecular structure (flavan-3-ols and their gallates), except grape seed constituents reach larger molecular sizes which are very poorly bioavailable. GSP markedly decreased membrane lipid peroxidation, recycled oxidized tocopherol in the membrane, and delayed hemolysis.

Other researchers have taken the view that it is unlikely polyphenols act as direct antioxidants under physiological conditions. As a result, nutrigenomics has emerged as a multidisciplinary area of research. Effects on different pathways can lead to changes in cellular function and ultimately health benefits. This field is not limited to polyphenols, but embraces phytochemicals as a whole.

On the other hand, some naturally occurring polyphenols are absorbed very well and a significant proportion is excreted in the urine. Examples include gallic acid and the isoflavones genistein and daidzein. To date, the highest concentrations of polyphenols observed in plasma following oral ingestion by humans have been in the 1–10 μM

range. These concentrations are only observed for relatively large doses of polyphenols from dietary supplements or polyphenol-rich foods and beverages that contain the more bioavailable flavonoids.

3.3. Anticancer Effect of Polyphenols

According to 2004 publication, approximately 12-25% Americans use herbal supplements. National Health and Nutrition Examination Survey (NHANES) data collected from 2003 to 2006 that covered all types of dietary supplements indicate that 53 percent of American adults took at least one dietary supplement; consumption among woman is generally greater than men. Polyphenols are increasingly receiving attention as dietary supplements, not only for scavenging free radicals, but as anticancer, antiviral, antimicrobial, vasorelaxant, anticoagulation, and anti-inflammatory agents. During chronic inflammation, polyphenolics may change key metabolic pathways. More generally, there is evidence some phenolic compounds target different intracellular pathways in a concentration-dependent manner. For example, low doses of red wine polyphenols promote angiogenesis via activation of the Akt/PI3K/eNOS, p38MAPK pathway, but not the NF-κB pathway. However, at high doses, they become antiangiogenic, inhibit the Akt/PI3K/eNOS pathway, and simultaneously enhance the NF-κB pathway. Other polyphenols are credited with having metal sequestering capabilities which may prevent the cytotoxic effects of heavy metal accumulation over time. Some common dietary polyphenols may inhibit the proliferation of cells, decrease angiogenesis, and modify cell signaling systems to initiate apoptosis.

4. Resveratrol

Resveratrol is a naturally occurring phytoalexin produced by plants in response to environmental stress or pathogenic attack. *trans*-Resveratrol has been identified in a wide variety of plants, including Japanese knotweed, peanuts, different kinds of berries, rhubarb, legumes, and grasses, but grapes and red wines are the main dietary sources. The compound was first reported by our group as a candidate for cancer chemoprevention. We found resveratrol acts as an antioxidant and antimutagen, induces phase II drug-metabolizing enzymes, mediates anti-inflammatory effects, inhibits cyclooxygenase and hydroperoxidase functions, and induces human promyelocytic leukemia cell differentiation. Together with short-term animal studies, it was concluded the compound had potential to inhibit carcinogenesis at the stages of initiation, promotion, and progression. Numerous studies have followed; a plethora of activities have been demonstrated. A few examples include down-regulation of the synthesis and release of proinflammatory mediators, inhibition of activated immune cells, aromatase, inducible nitric oxide synthase (iNOS), cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), and inhibition of NF-κB. Although one high-affinity target has been identified, quinone reductase 2 (QR2), it does not appear likely that resveratrol functions through one specific mechanism.

In addition to cancer chemoprevention, laboratory and animal studies have shown that resveratrol may help to prevent heart disease and many other human ailments. However, the actual impact of resveratrol on human health, as a dietary component or supplement, still remains moot. Questions involving relatively low potency, pleiotropic mechanisms,

and rapid metabolism are unresolved. Human studies suggest daily doses in the range of 1–5 g are safe and acceptable for clinical trials. Of course dosage levels in this range are not possible through normal dietary consumption, so resveratrol is evolving more as a natural product drug than a natural dietary component that may affect human health.

4.1. Antioxidant Activity of Resveratrol

Many of the thousands of investigations reported with resveratrol have focused on antioxidant, antiinflammatory, and metal-chelating properties. Reactive oxygen species (ROS) can damage proteins, nucleic acids, and membrane polyunsaturated fatty acids, and destroy membrane integrity and reduce mitochondrial membrane potential. Data has been generated indicating that resveratrol inhibits lipid peroxidation mainly by scavenging lipid peroxyl radicals within the membrane (similar to vitamin E). The capacity of resveratrol to enter the lipid environment accentuates antioxidant potential. We demonstrated resveratrol can inhibit 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced free radical formation with cultured HL-60 cells. Resveratrol inhibited H_2O_2-induced DNA damage in human lymphocytes by increasing glutathione levels, induction of glutathione peroxidase, glutathione reductase, and glutathione *S*-transferase.

Many polyphenolic compounds from fruits and vegetables, including resveratrol, are known for antioxidant properties, and some studies suggest promise for protection against cardiovascular disease. Skin cells may also be subjected to high oxidative stress, suggesting resveratrol may be of value for skin aging and skin cancers. In the central nervous system, neurons are vulnerable to neurotoxins, leading to ischemia, stroke, and seizure. There are some suggestions of resveratrol having value for the treatment or prevention of neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, and stroke. In addition to counteracting ROS production, there is growing evidence that resveratrol may act as a pro-oxidant and induce apoptosis in cancer cells. The antioxidant and pro-oxidant activities of resveratrol may induce differential effects, depending on the cell type ("normal" and cancer cells), cellular conditions (normal or malignant), and concentrations.

One interesting hypothesis relates to intracellular levels of copper. *trans*-Resveratrol does not chelate iron, but efficiently chelates copper. In cells with elevated copper levels, such as cancer cells, a strong pro-oxidant effect can be generated in the presence of resveratrol. This, in turn, can lead to DNA damage. Therefore, the ability to regulate antioxidant and pro-oxidant activities of resveratrol in the presence of copper may be a new strategy for modulating cytotoxicity and induce apoptotic activities in neoplastic cells.

4.2. Resveratrol and Cancer

Thus far, no appreciable toxicity has been demonstrated with resveratrol, even with doses as high as 5 g/day. Consumption of a resveratrol-rich grape supplement improves the inflammatory and fibrinolytic status in patients who were on regimens for the prevention of CVD. Some reports show that consumption of resveratrol may interfere with blood thinners increasing the risk of bleeding, so people with heart/blood vessel

disease and on blood thinners may still consider taking a supplement with phenolic compounds, including resveratrol for improving their heart health.

Considering the cancer chemopreventive potential of resveratrol, as demonstrated in our original report, this is especially important. In addition to observing activity against the three major stages of carcinogenesis (initiation, promotion and progression), our original report illustrated significant anti-inflammatory activity in the rat paw model. It is widely accepted that chronic inflammation can lead to various cancers, so inhibition of inflammation is a promising approach for cancer chemoprevention. Many studies have subsequently confirmed the anti-inflammatory potential of resveratrol. For example, it can suppress activation of nuclear transcription factor NF-κB that regulates the expression of various genes involved in inflammation, cytoprotection, and carcinogenesis. It inhibits expression of proinflammatory cytokines, such as TNF, IL-1, IL-6, and IL-8, and abrogates the expression of proteins mediating inflammation such as iNOS, COX-2, and 5-LOX.

Resveratrol has been shown to suppress the proliferation of a wide variety of tumor cells, including lymphoid and myeloid cancers as well as cancers of the breast, prostate, stomach, colon, pancreas, and thyroid. Resveratrol inhibits cell proliferation by blocking cell-cycle progression in different phases: G1, S, S/G2, or G2. Why the effects of resveratrol vary so widely with different cell types requires further investigation. In addition to inhibiting proliferation, resveratrol induces apoptosis either through the caspase 8-dependent and receptor mediated pathways (type I) or the caspase 9-dependent pathway, mediated through mitochondria (type II), or both.

In vitro studies of these types suggest resveratrol holds promise for the prevention or treatment of cancer. However, although a few clinical studies have been performed with human beings, and further studies are under way, efficacy has yet to be demonstrated. One major issue is oral bioavailability. Although resveratrol is well absorbed on oral administration, metabolism is rapid and near complete. Since resveratrol possesses a simple chemical scaffold with multiple potential manipulation sites, chemical modification is one approach to improving biopotency and bioavailability. A simpler approach is to consider topical applications for skin disorders. Several topical formulations of resveratrol are being developed, including some using stabilizers and surfactants to produce a stable nanosuspension of resveratrol.

4.3. Resveratrol Metabolism and Pleiotropic Effects

An unusual feature of resveratrol is that it acts through numerous mechanisms, including regulation of cell cycle progression, apoptosis, inhibition of tumor invasion and angiogenesis, prevention of inflammation, scavenging of ROS, and modulation of NF-κB. Considerable attention was generated when resveratrol was publicized to activate a histone deacetylase (SIRT1), but studies performed with greater experimental agility revealed this was not correct. Presently, the ability and usefulness of resveratrol to extend lifespan and function as a mimic of caloric restriction remains moot.

In any case, the regulation of multiple anti-cancer pathways (pleiotropism) can be viewed as an attractive feature since it may help to overcome drug resistance. As a

corollary, due to this multifaceted action, resveratrol has been touted as a treatment for numerous human ailments. A confounding factor is that resveratrol is readily absorbed in human beings but rapidly metabolized into sulfate and glucuronide conjugates; the concentrations necessary to mediate activity observed with *in vitro* systems cannot be achieved in animals. In turn, since serum concentrations of sulfated metabolites are higher than the serum concentrations of resveratrol, the potential of metabolites to mediate biological responses has been investigated. In general, the metabolites are less active than the parent molecule, although some exceptions are known. For example, compared to resveratrol, we have shown resveratrol 3-sulfate mediates comparable or even greater QR1 induction, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, and COX-1 inhibition. Therefore, interplay is possible between resveratrol and its metabolites that results in an overall response.

From a drug development point-of-view, as a promiscuous molecule that interacts with numerous targets, resveratrol can be viewed as a scaffold for designing structural relatives potentially capable of mediating more intense responses with greater mechanistic stringency. We synthesized and evaluated scores of resveratrol analogs, and several compounds were found to mediate responses with much greater potency and specificity. In addition, when administered to rats, higher serum concentrations and greater stability were demonstrated with prototype lead molecules. These data support the promise of more advanced development of novel resveratrol derivatives as drug entities. Another advantage is the availability of facile syntheses for large-scale manufacture since the structures are not overly complex.

5. Chemistry of Wine

Over 50 percent of the general population in western countries has a chronic condition such as high blood pressure, arthritis, diabetes, asthma, or osteoporosis. Flavonoids and phenolic acids have antibacterial, antifungal, antineoplastic, hepatoprotective, immunomodulating, and antiinflammatory properties. They are used for allergies, asthma, diabetes, hypertension and cancer; and their pharmacological effects are generally associated with antioxidant activity. One of the most abundant sources of polyphenols, mainly flavonoids and phenolic acids, is grapes and wine. Phenolic compounds in grapes and wine are represented by following major classes: stilbenes, phenolic acids, ellagitannins, anthocyanins, and flavonols. Phenolics can be glucuronidated, sulfated, and/or methylated following phase II conjugation metabolism. The majority of phenolic compounds from grapes and wine are metabolized in the gastrointestinal tract by gut microflora. Some health benefits resulting from grape and wine consumption may be due to these metabolites.

Some epidemiological studies have suggested moderate consumption of wine reduced mortality due to a lower incidence of coronary heart disease and cancer. Wine consumption has been associated with an inverse association with colorectal cancers, and light wine intake could even protect against nonalcoholic liver disease. On the other hand, high consumption of wine may pose certain health risks. Alcohol is linked with an increased risk of cancer of the mouth, esophagus, pharynx, larynx, and breast cancer in women. However, the cardiovascular benefits of moderate drinking (2 drinks a day for

men, 1 drink a day for women) may outweigh the risk of cancer. Polyphenols in wine (Figure 2) may be responsible for some of these effects.

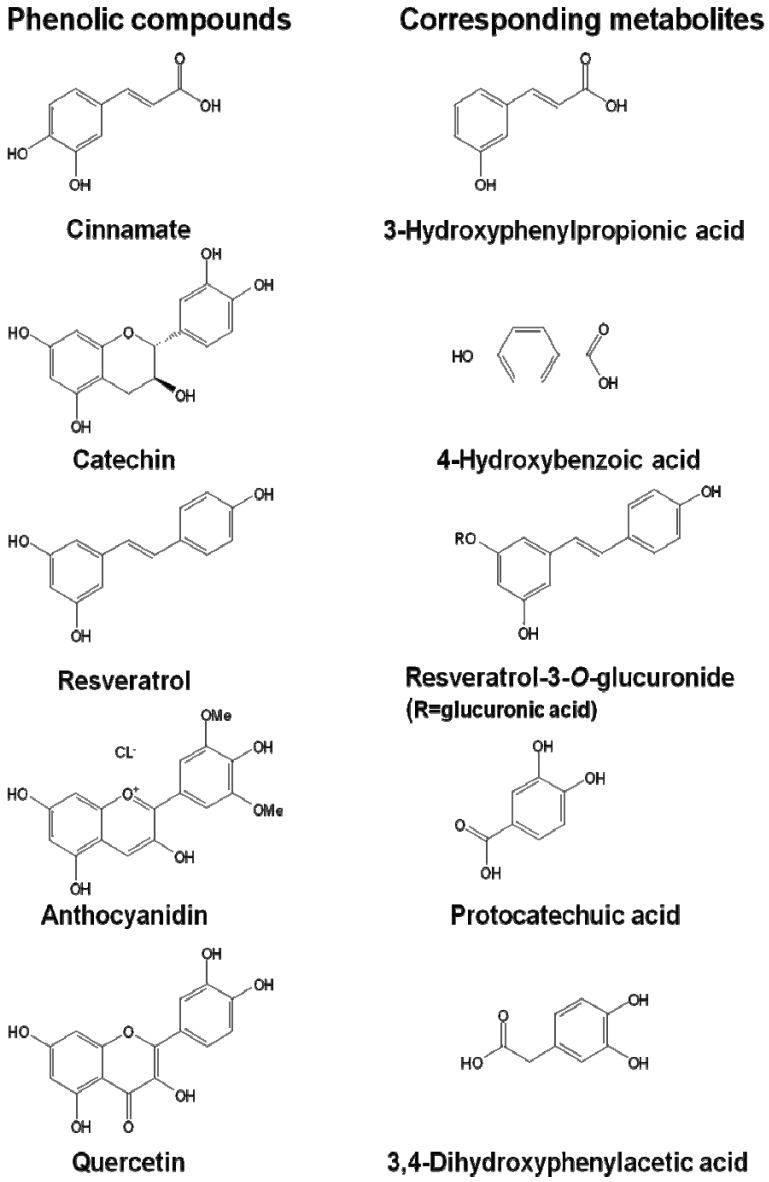

Figure 2. Selected examples of phenolic compounds and their representative metabolites in wine.

The majority of the potentially bioactive compounds in wine are also found in grapes. For instance, anthocyanins found in cabernet sauvignon, merlot, and syrah grapes are effectively extracted into their wines. It is not clear, however, if bioavailability is the same if ingested as a component of grapes or wine. Matrix effects may play a significant role in the absorption of grape and wine phenolics and, once absorbed, most grape and wine flavonoids are rapidly metabolized, making it difficult to determine whether these compounds are effective against particular diseases. In addition, phenolic acid metabolites are formed from gut microflora. There is a multitude of grape and wine

phenolic metabolites (Figure 2), so it is important to identify and quantify specific chemical entities in plasma, urine, and feces.

Some resveratrol producing plants, including berries and wine grape, are part of the human diet. In the grape, resveratrol is found in the skin but not the flesh. This has a bearing on the amount ultimately found in wine. In general, the amount of resveratrol in red wine is significantly higher than white wine. Quantities vary in different red wine grades; some Pinot noirs show the highest concentrations. Red wine is one of the richest sources of polyphenols in the human diet. Highly tannic red wines can contain up to 3 g of polyphenols per liter, and moderate red wine drinkers consume polyphenols at levels well above the population average.

The possible interaction of resveratrol with other red wine constituents or other cancer therapeutics has to be taken in account as well. Antiplatelet effects of prostaglandins are potentiated by low concentrations of resveratrol. With human leukemic cells, ellagic acid and quercetin interact synergistically with resveratrol in the induction of apoptosis. Resveratrol improved the anti-cancer efficiency of doxorubicin, but also enhanced doxorubicin-induced bradycardia. *In vitro* assays showed that the anti-radical activity of resveratrol was reduced in the presence of quercetin and catechin.

A number of studies have investigated the effect of grape beverages on endothelial function. Consumption of purple grape juice for 2-4 weeks improved brachial artery flow-mediated dilation in patients with coronary artery disease. Dealcoholized wine had the same effect in healthy subjects. Red wine consumption prevents the impairment of endothelial function that occurs in heavy cigarette smokers. Catechins and resveratrol increase protein levels and the activity of tissue plasminogen activator, suggestive of cardioprotective activity. Polyphenols in red wine affect endothelial regulation of inflammation. Thus, grape polyphenols appear to induce many favorable changes in endothelial cells that could reduce cardiovascular risk in human beings.

6. Concluding Remarks

Grapes and wine have been consumed since ancient times, and some health benefits have been associated with these dietary practices. Cardiovascular diseases have been studied most intensively, but many other disease states have been considered as well. Potential benefits of moderate wine consumption gained significant attention due to revelation of the ‘French Paradox.’ However, since we reported the cancer chemopreventive potential of resveratrol, a stilbene uniquely associated with grapes as a dietary component, the field has burgeoned. Interestingly, however, despite thousands of scientific investigations, the actual impact of resveratrol on human health remains moot. It has relatively low potency, facilitates pleiotropic mechanisms, and is rapidly metabolized. The phenomenon of resveratrol-induced SIRT1 activation achieved a high level of notoriety but was subsequently proven to be incorrect. Clinical trials will be required to establish potential of resveratrol to mediate any health benefits. Improved formulations or synthetic analogs may provide better results and be of greater value in the future.

Perhaps of more general interest, grapes and wine are comprised of scores of compounds, all of which are ingested by human beings. Strong evidence suggests health

benefits may be derived from the holistic product. In studies performed under the auspices of the California Table Grape Commission, using whole-grape powder as a surrogate for fresh grapes, positive results have been obtained in a number of areas. Examples include prevention of photodamage to skin, protection of urinary bladder function, protection of healthy colon tissue against cancer by inhibiting target genes that promote colon cancer, attenuation of inflammation in human macrophages, prevention of vision loss in animal models of age-related blindness, protection of neuronal cells in a gerbil model subjected to ischemia, and attenuation of atherosclerosis development in apolipoprotein E deficient mice. Additional work is underway in these and many other areas.

Grape seed extract is highly promoted largely due to strong antioxidant activity. As with many natural products, open questions remain regarding absorption and metabolism, and it is not clear if adequate serum concentrations can be achieved to benefit by antioxidant effects. However, indirect effects are possible, such as induction of phase II enzymes or nucleophiles such as glutathione, and there is interplay between antioxidant and pro-oxidant mechanisms. Pro-oxidant activity may be viewed as a double-edged sword, depending on the site of activity. Although apoptosis may be induced in transformed cells, genotoxic effects may be provoked in normal cells.

Altogether, examination of compounds associated with grapes is a worthwhile endeavor that may help us understand new mechanisms of action. Structural diversity is remarkable and biological activities have been reported with numerous experimental systems. Of the scores of minor chemical components present in the grape, it is likely that unique activities will continue to be discovered and investigated. Similarly, as has been the case with resveratrol, structure-activity relations will be explored and chemical derivatives with greater efficacy and specificity will be produced. Beyond positive organoleptic experiences, it is apparent that modern-day society is intrigued by healthy foods and dietary habits associated with maintaining or promoting good health. At this point in time, consumption of whole grapes or grape products has the greatest influence on human health. Grape-derived nutraceutical products are available, but consumers should carefully scrutinize health-related claims and innuendo, and ultimately rely on data obtained in properly conducted clinical trials.

Glossary

Angiogenesis **:** An important process in the body related to the growth of new capillary vessels

Anthocyanidins **:** Are sugarless plant pigments responsible for color

Antioxidant **:** Antioxidant agents interact with oxidants and reduce harmful effects

Apoptosis **:** Programmed cell death

Bioavailability **:** The amount of or rate at which a substance is accessible to the body

Biomarkers **:** Are key molecules or cellular events that that are indicative of a broader health condition

Biopotency **:** A measure of the ability of a substance to have a biochemical

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Biographical Sketches

Dr John M. Pezzuto received his B.S. degree in chemistry from Rutgers University (1973), and Ph.D. in biochemistry from the University of Medicine and Dentistry of New Jersey (1977). He performed postdoctoral work in the Department of Chemistry at Massachusetts Institute of Technology, followed by a one-year stay at the University of Virginia as Instructor of Chemistry. He served on the faculty at the University of Illinois at Chicago (1980-2002), rising through the ranks to Distinguished University Professor. He serves as department head, Associate Director of the Cancer Center, Director of the Program for Collaborative Research in the Pharmaceutical Sciences, and Associate Dean for Research and Graduate Education. In 2002, Dr. Pezzuto accepted the position of Professor of Medicinal Chemistry and Molecular Pharmacology and Dean of the College of Pharmacy, Nursing and Health Sciences, at Purdue University, West Lafayette, Indiana. He served as Professor and Founding Dean of the College of Pharmacy at the University of Hawaii at Hilo (2006-2015), and currently serves as Professor and Dean of the College of Pharmacy at Long Island University, Brooklyn, NY. He is an author of over 500 publications, co-inventor of several patents, the editor of three books, member of eleven editorial boards of international journals, the former editor-in-chief of the International Journal of Pharmacognosy (1990-1995), the former editor-in-chief of Combinatorial Chemistry and High Throughput Screening (1996-1997), and the current editor-in-chief of Pharmaceutical Biology. He was the recipient of a Research Career Development Award from the National Cancer Institute (1984-1989) and a Research Fellowship from the Alexander von Humboldt Foundation (1990-1991). He has directed the research of numerous doctoral students, postdoctoral associates, and visiting scholars. He was elected Senior University Scholar at UIC in 1999, and UIC Inventor of the Year in 2000. His current research interests are predominately in the areas of biology-driven natural product drug discovery and characterization, with primary emphasis in the fields of cancer chemotherapy and cancer chemoprevention.

Dr Tamara P. Kondratyuk is an Assistant Specialist in the Department of Pharmaceutical Sciences, College of Pharmacy, University of Hawaii at Hilo. She holds Ph.D. in Biochemistry; she received her postdoctoral training from Purdue University in West Lafayette, Indiana and joined Natural Product Drug Discovery Lab, PI Dr. John M. Pezzuto since then. Her work led to the discovery of many biologically active compounds from plants, marine microorganisms, synthetic compounds, and her research results can be found in many peer-reviewed publications. As a researcher on several NIH-funded grants, she is aware of the importance of cancer chemoprevention natural products discoveries and believes that her expertise will help to contribute to the ongoing success of these studies.