RESVERATROL AND OCULAR DISEASE PREVENTION

Dominique Delmas - France

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SUMMARY

AUTHOR

Dominique Delmas, has obtained his Ph.D. in Biochemistry, Cellular and Molecular Biology at the University of Burgundy (France) on December 16th, 2002. His thesis entitled "Studies of a natural polyphenol, Resveratrol, on the proliferation, cell cycle and apoptosis of colon and hepatic cancer cells" focused on the antitumor effects of resveratrol. Then, from September 2002 to September 2004, he was appointed assistant teacher-researcher at the Faculty of Sciences of the University of Burgundy and in 2004, an assistant professor position in Biochemistry, Molecular and Cellular Biology at the University of Burgundy. In 2009, he obtained his Research Management Habilitation and was rewarded twice by the French Research Ministry with the Scientific Excellence Price (PES). Currently, Dominique Delmas is charge with the biochemistry teaching of the 3rd level of Biochemistry Bachelor's degree. He is an elected member of the University of Sciences of Burgundy board, and he is also member of Scientific Societies such as the French Society of Biochemistry and Molecular Biology (SFBBM), the Federation of European Biochemical Societies (FEBS).

In the Research Center Inserm Unit U866 "Lipids, Nutrition, Cancers" in Dijon, Dominique Delmas manages a research group within the Inserm team "Chemotherapy, Lipid Metabolism and Antitumoral Immune Response" where he supervises post-doctorants, PhD and Master students.

The main research objectives of his group are to develop and to coordinate multidisciplinary and translational research from laboratory to clinical applications, including 1) the determination of early biomarkers in response to treatment thanks to the analysis of membrane dynamic and of the associated signaling pathways; 2) the identification of new potential targets within membrane and lipid metabolism, 3) the development of new therapeutic strategies by the use of molecules with low toxicity (i.e. polyphenols) that potentially modulate the tumoral micro-environment. He is currently involved in collaborative projects with various research teams in France and abroad. His research is also oriented towards industrial transfer by developing research programs in which he determines the health potential of natural molecules / extracts or synthetic molecules by a) studying their molecular and cellular mechanisms of action in vitro and in vivo, by b) studying the initial membrane events in particular those linked to lipid rafts, by c) determining the structure-activity relationships and the bioavailability of these compounds. In this area, he supplies also a scientific support in the fields of inflammation and lipid metabolism.

Dominique Delmas has authored or coauthored over 30 original papers and comprehensive reviews on polyphenols health effects. He has also contributed to over 10 books chapters concerning the molecular mechanisms of polyphenols in tumoral cells. Furthermore, he has recently participated to an expert committee, held in September 2010 in Helsingor (Denmark), which brought him to publish in PlosOne his recommendations and future directions for the coming years in the resveratrol research field.

INTRODUCTION

Numerous epidemiological studies have suggested that environmental factors, such as metabolic diseases or air pollution, could contribute to various ocular diseases. On the contrary, antioxidants as vitamin E or fatty acids, especially polyunsaturated acids (PUFA), could be used as supplementation to protect against diseases such as ocular diseases through their antioxidant power (Figure 1). In this way, the Age Related Eye Disease Study 1 (AREDS-1) a multicenter, randomized controlled clinical trial demonstrated that oral nutritional supplementation of a combination of vitamin C (500 mg), vitamin E (400 UI), β-carotene (15 mg) zinc oxide (80 mg) and cupric oxide (2 mg) in patients with intermediate or advanced Age-related Macular Degeneration (AMD) in one eye had a 25 % relative risk reduction over 5 years of developing advanced AMD. The risk of vision loss of three or more lines was reduced by 19 % with this supplementation. Moreover, several epidemiological studies demonstrated that carotenoid intake reduced the risk for advanced AMD and that lutein and zeaxanthin based diet might protect against intermediate AMD female patients. In this way, the objective of the second AREDS (AREDS-2) was to determine whether the addition of lutein/zeaxanthin and omega-3 fatty acids (docosahexenoic [DHA] and eicosapentenoic acids [EPA]) would further reduce progression to late-stage AMD. The addition of lutein + zeaxanthin, DHA + EPA, or lutein + zeaxanthin and DHA + EPA to the complete AREDS formulation did not further reduce the risk of progression to advanced AMD[1, 2]. Moreover, because a potential increased incidence of lung cancer in formers smokers, lutein + zeaxanthin could be an appropriate carotenoid substitute in the AREDS formulation[2].

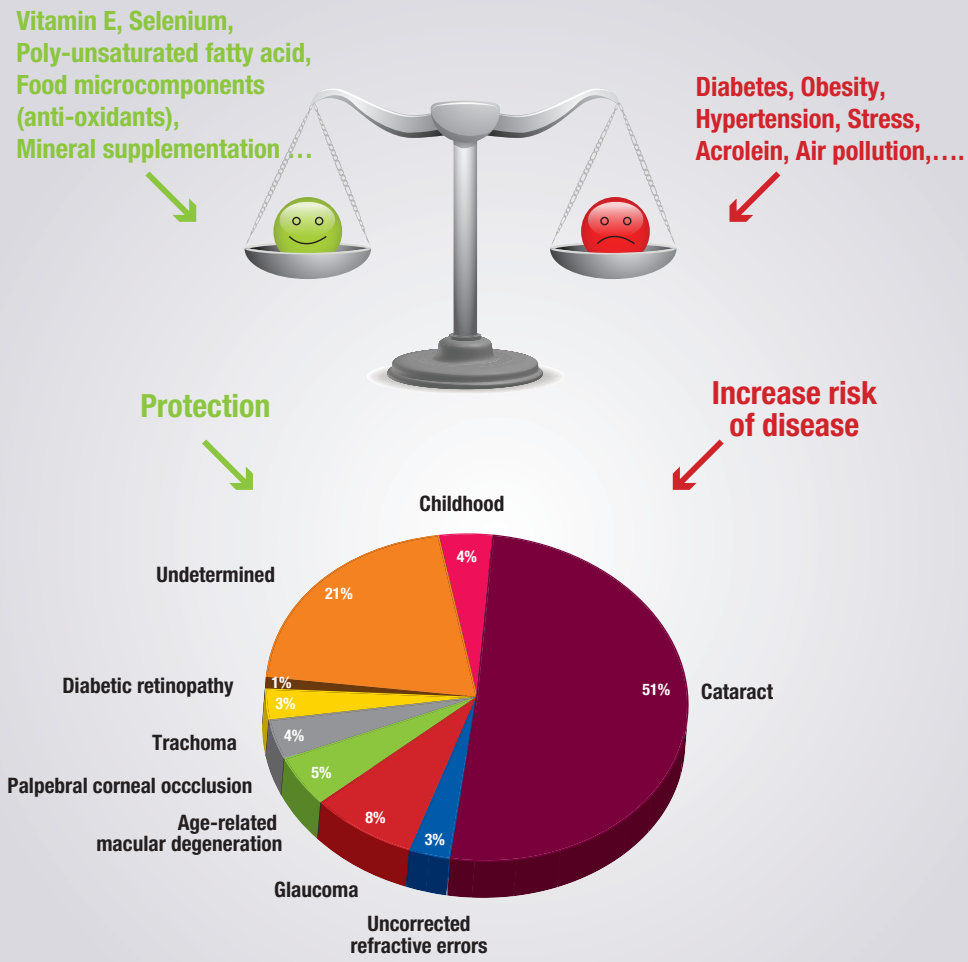


FIGURE 1. **Environmental factors involved in protection or in increase risk of ocular diseases** (adapted from Delmas, EVER Congress 2013).

Nevertheless, among the microcomponents of the diet which can participate to the protection of the ocular diseases, other bioactive compounds could be good candidates, particularly polyphenols (Figure 2). Indeed, various studies have shown that polyphenols may protect against vascular diseases, cancers and associated inflammatory effects. These phytomolecules have cellular targets similar to those of the new drugs developed by pharmaceutical companies. Indeed, more than 1,600 patents are currently reported concerning flavonoids and 3,000 patents concerning polyphenols.

Pleiotropic pharmaceutical activities are claimed in fields such as cancer, inflammation arthritis, eye diseases and many other domains. One of the best known is the polyphenol resveratrol which is a trans-3,4',5-trihydroxystilbene (resveratrol) and seems appear to a great interest in the prevention of these pathologies. Resveratrol presents a myriad of health benefit effects and acts at multiple levels such as cellular signaling, enzymatic pathways, apoptosis and gene expression to prevent or to fight coronary heart damages, cancers or degenerative diseases.

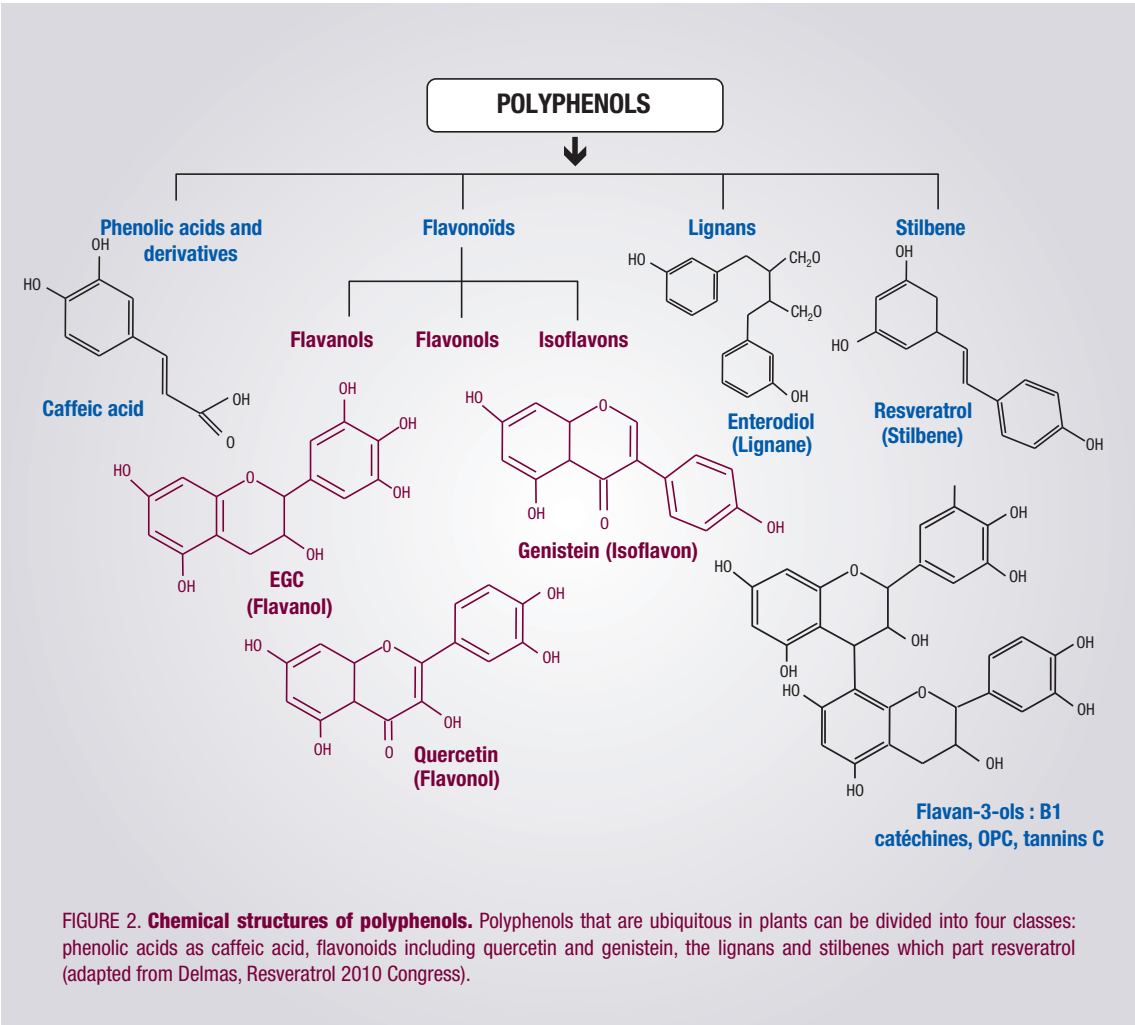


FIGURE 2. **Chemical structures of polyphenols.** Polyphenols that are ubiquitous in plants can be divided into four classes: phenolic acids as caffeic acid, flavonoids including quercetin and genistein, the lignans and stilbenes which part resveratrol (adapted from Delmas, Resveratrol 2010 Congress).

CHAPTER 1

RESVERATROL

1. THE ORIGINS OF RESVERATROL

Resveratrol is a secondary metabolite produced in limited plant species. The root of the word «resveratrol» is a combination of the latin prefix Res, meaning «which comes from», *veratr,* from the plant «Veratrum», and the suffixe ol, indicating that it contains «alcohol» chemical groups. *Veratrum grandiflorum* has been reported to synthesize resveratrol and analogues. It is interesting to note that root powder of *Veratrum album* has long been used for at medium altitude in Northern Europe, Asia and Japan to treat rheumatisms and nervous diseases. However, *Veratrum album* contains potent toxic alkaloids: the protoveratrines A & B. The resveratrol precursor is phenylalanine and the key cell enzyme is stilbene synthase which orientates the synthesis pathway toward resveratrol, instead of toward flavonoids through chalcone synthase [3]. Therefore, resveratrol can be classified either as a stilbene or as a polyphenol (Figure 2).

First, a significant amount is found in the leaves when the plant is damaged by chemical treatments [4] ; and secondly, the roots and rhizomes are relatively rich in resveratrol, and as such were used as crude preparation to treat hypertension in the East [5]. One of the richest sources of resveratrol is Japanese knotweed *(barnbuo japonaise) Polygonum cuspidatum*, root extract. These roots play an important role in ancient Chinese and Japanese natural medicine [6, 7]. Its presence has been reported in many trees including eucalyptus [8, 9], the spruce [10] and tropical trees like *Bauhinia racemosa* [11]. Resveratrol has been identified in a few flowering plants, only two species of hellebore, *Veratrum grandiflorum* and *Veratrum formosanum*, are able to synthesize this compound. Resveratrol is also present in *Pterolobium hexapetallum*, a legume [12]. Cotyledons such as peanut *Arachis hypogaea* synthesize a set of stilbene - phytoalexines including resveratrol with concentrations significantly increased in response to infection, injury and UV irradiation [13-17].

Tableau 1. Trans-resveratrol concentrations in various food sources (adapted from Delmas, 2009, HDR, Université de Bourgogne)

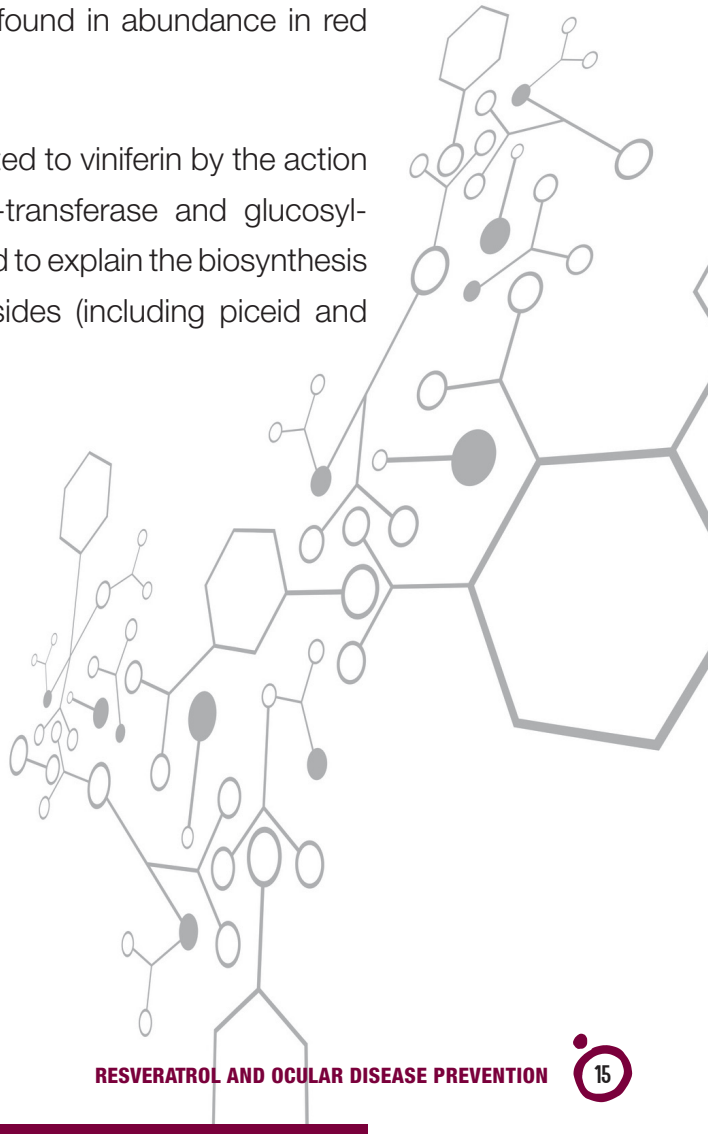
Sources	*Trans*-resveratol (µg/g)	References
Hop	0.5	[18]
Peanuts	5.1	[19]
Peanut butter	0.3	[19]
Grape skin	27.5	[20]
Ko-Jo-Kon	523	[19]
Blueberries	0.03	[21]

Beverages	*Trans*-resveratol (µg/g)	References
Pinot noir (red)	10.6	[19]
Gamay (red)	28.3	[22]
Régent (red)	10.0	[22]
Gamay (rosé)	4.8	[22]
Chardonnay (white)	0.43	[22]
Chasselas (white)	0.86	[22]
Ko-Jo-Kon (infusion)	0.68	[19]

The importance of resveratrol in plant biology lies in its ability to inhibit the growth of fungal infection, a property that has allowed it to be included in the class of antibiotic plants: phytoalexin. This interest in resveratrol mainly concerns vines *(Vitaceae)* in reason of its function as a phytoalexin and its role as a marker of infection by various pathogens. The first reports describing the presence of resveratrol in grapes and its induction by infection with a fungus such as *Botrytis cinerea* [23] shows that this hydroxystilbene can exist in two forms: *trans*-resveratrol, the most organic form active blocking the development of *Botrytis cinerea*, and *cis*-resveratrol obtained by the action of light on the *trans*-form.

Most grape varieties occur in varying quantities this natural fungicide molecule, the grape skin containing about 50 to 100 µg of resveratrol per gram. It follows that during maceration, which takes place in the red wine vinification, resveratrol is dissolved in the hydro-alcoholic medium and then found in abundance in red wine up to 20 mg/l[24].

Furthermore, resveratrol can be converted to viniferin by the action of vacuolar peroxidases [25, 26]. Methyl-transferase and glucosyl-transferase activities have been identified to explain the biosynthesis of pterostilbene and resveratrol glucosides (including piceid and resveratrol-3-O-β-D-glucoside) [27-29].



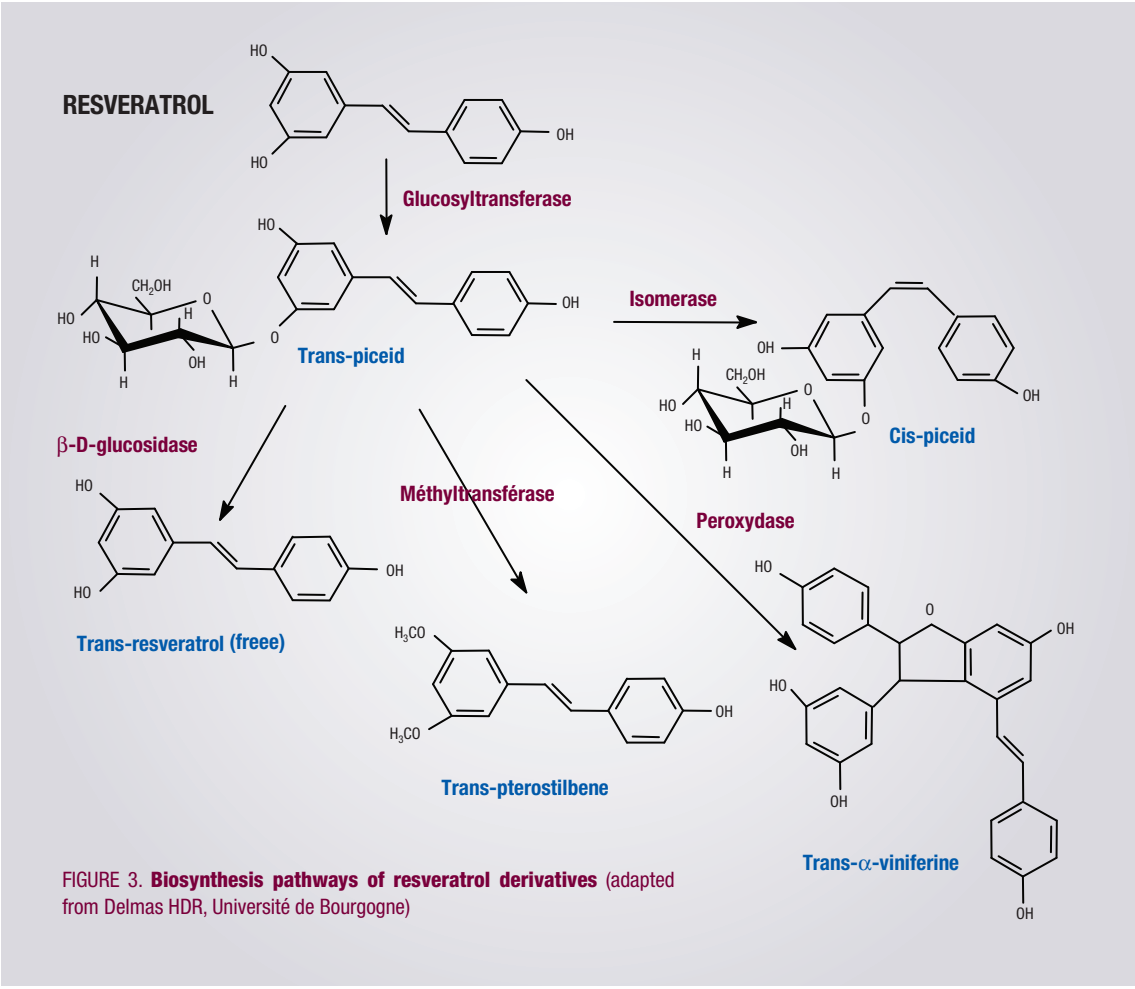


FIGURE 3. **Biosynthesis pathways of resveratrol derivatives** (adapted from Delmas HDR, Université de Bourgogne)

Like many other plant polyphenols, resveratrol is considered as a preventive food microcomponent in a similar manner as the flavonoids and epicatechins of green tea or cocoa [30]. In fact, resveratrol attracted little interest until 1992 when it was postulated to explain some of cardioprotective effects of red wine and then increased from 1997 when Pezzuto's team published a seminal paper reporting the ability of resveratrol to inhibit carcinogenesis at multiple stages [31]. Since this date, the number of papers and citations were increased in exponential manner due to the pleiotropic effects of resveratrol on various diseases (Figure 4).

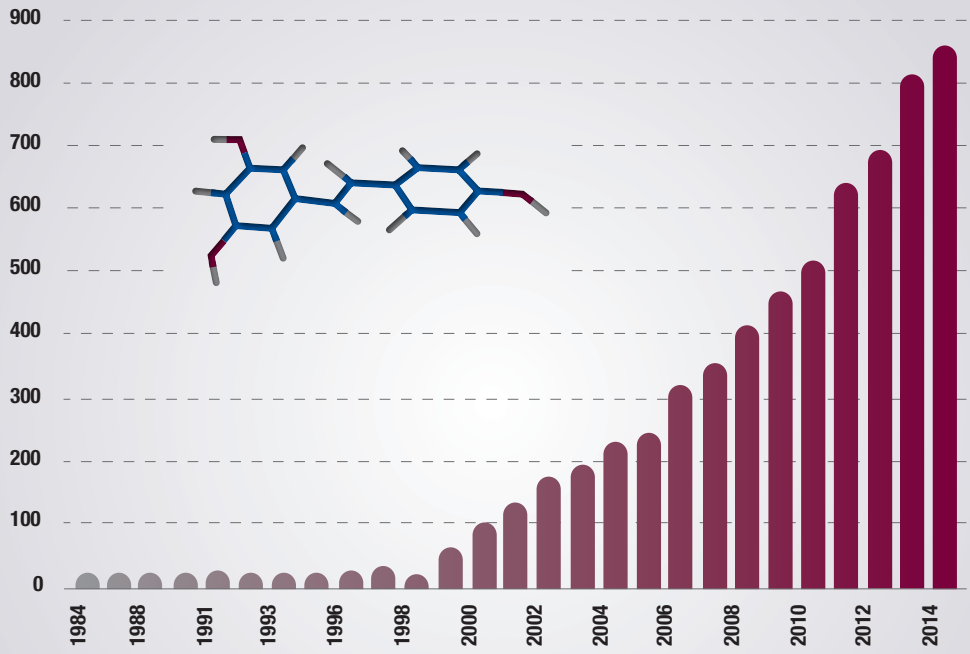


FIGURE 4. **Resveratrol citations appearing on PubMed as a function of year.** The PubMed database was searched using the key words « resveratrol ». The bars show the cumulative number of hits identified for each year after the creation of Medline in 1963.

2. RESVERATROL AND CARDIOPROTECTIVE EFFECTS

Some reports have shown that regular consumption of red wine is often credited as the explanation for the "French Paradox" [32] – a term coined to describe the observation that the French enjoy a relatively low risk of cardiovascular disease despite a diet that is high in saturated fat (Figure 5) [33]. The discovery that resveratrol is obtained primarily from red wine in most human diets has prompted extensive research into its potential to explain these cardioprotective effects.

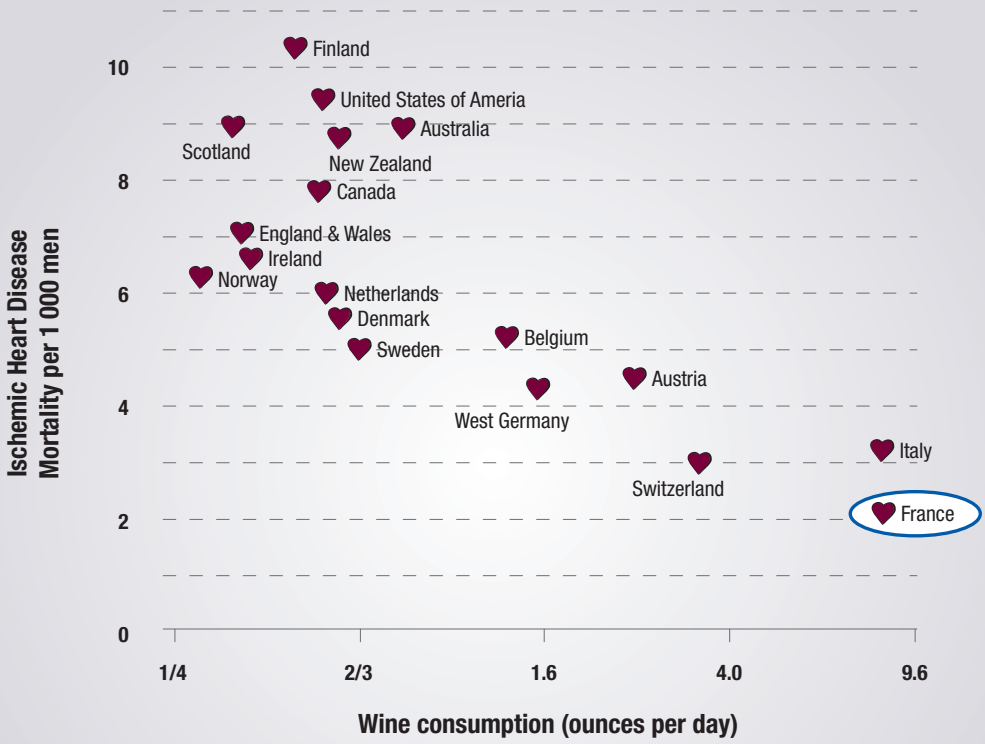


FIGURE 5. **Wine consumption effect on ischemic heart diseases mortality.** An inverse relation appears between average per capita wine consumption and mortality from coronary heart diseases in 18 western countries (adapted from Golberg *et al.*, Clinica Chimica Acta, 1995).

Indeed, the first biological properties of resveratrol have concerned its antioxidant power that can contribute to prevent coronary heart diseases (CHD) particularly atherosclerosis (Figure 6).

The atherosclerotic process is the result of disruption of normal reactions between the blood (plasmatic proteins, lipoproteins, growth factors, lymphocytes, platelets) and the normal cellular elements of the arterial wall. An important event in the lesion formation is low-density lipoproteins (LDL) oxidation in the intima[34, 35]. Lipid peroxidation is a chain reaction process which can be induced by different free-radical sources (ionizing irradiation, UV light). Several groups have reported that oxidized-LDL (oxLDL) can stimulate platelet aggregation [36] or promote procoagulant activity in the surface of human monocytes / macrophages by an increase in tissue thromboplastin activity [37] or by stimulating the expression and secretion of the tissue factor by monocytes or aortic endothelial cells [38]. Oxidation of LDLs favors the transformation of macrophages into foam cells. The development of foam cells that contain massive amounts of cholesterol ester is a hallmark of both early and late atherosclerotic lesions. OxLDL derived cholesterol brought into the macrophage via scavenger receptors consists of free cholesterol as well as cholesterol esters that are hydrolyzed in lysosomes. In addition, oxLDLs stimulate endothelial cells (ECs) to produce chemokines, granulocyte and macrophage colony-stimulating factors, and they have direct chemotactic activity for monocytes to endothelium. Moreover, oxLDLs can also promote the proliferation of the smooth muscle cells (SMC) which are in part of resident intima cells that preceded the lesions, and in part their progeny that arose as a response to various stimuli (e.g. lipid accumulation, disruption of intima structure). Intima SMC accumulates large amounts of cholesterol esters and become foam cells. Furthermore, platelets contribute to the rate of development of atherosclerosis and coronary heart diseases through several mechanisms, particularly synthesis of pro-inflammatory molecules.

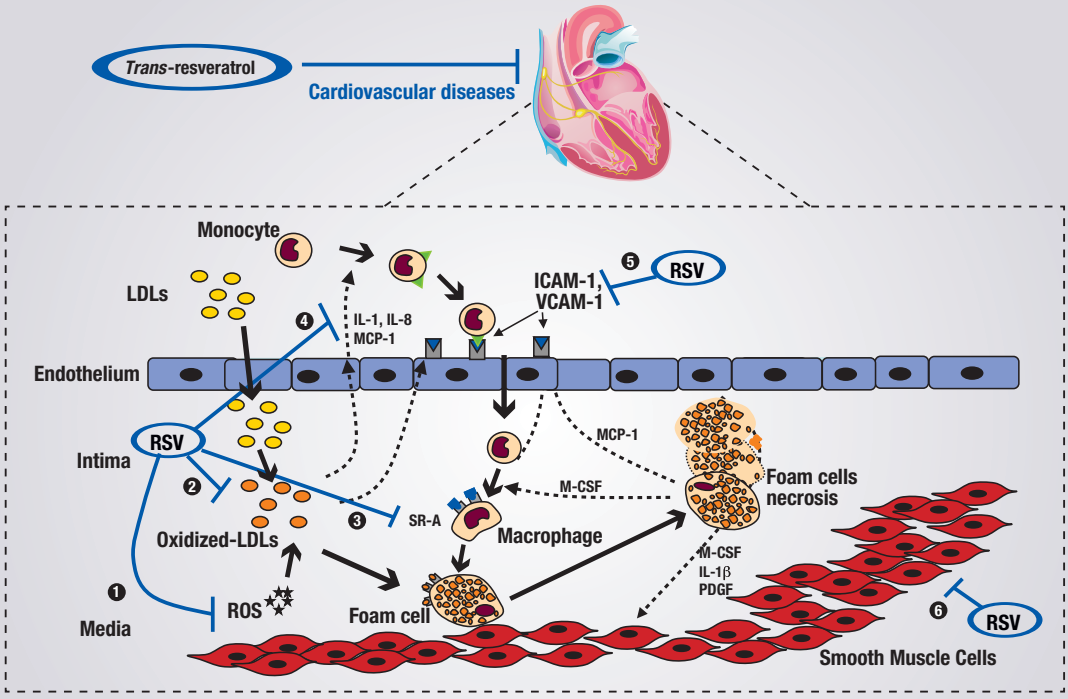


FIGURE 6. **Biological effects of resveratrol on atherosclerosis.** Resveratrol (RSV) prevents the initial events by 1) scavenging the ROS (*), by 2) reducing the oxidation of LDLs, by 3) dowregulation of scavenger receptor (SR-A) stimulated by several factors, by 4) inhibition of chemokine and cytokine production, by 5) decreasing the expression of ICAM-1 and VCAM-1 on endothelial cells, and by 6) reducing the SMC proliferation and migration (adapted from Aires and Delmas, Nova Publisher eds, 2013).

Resveratrol could act on the different steps of atherosclerosis:

2.1. Resveratrol scavenges the reactive oxygen species (ROS)

Frankel *et al.* were the first to demonstrate that resveratrol reduced the oxidation of human low density lipoproteins (LDL) induced by their incubation with a metal ions such as copper [39]. This effect should be assigned to the chelation of copper because metals act as pro-oxidants by electron transfer, releasing free radicals from polyunsaturated fatty acids and hydroperoxides. It has been demonstrated that resveratrol suppresses lipid peroxidation both by chelation of copper [40-43] and by scavenging of free radicals [40, 41, 44].

2.2. Resveratrol prevents the oxidation of low density lipoproteins (LDL)

Various enzymatic systems present in endothelial cells (ECs) or macrophages are implicated in the oxidation of LDL (Figure 6). Resveratrol can reduce LDL oxidation by modulating these enzymatic systems that are i) nicotinamide adenine dinucleotide (NADPH)-dependent oxidases [45], ii) hypoxanthine / xanthine oxidase (HX/XO) [46], iii) 15-lipoxygenase (15-LO), iiii) myeloperoxidase (MPO) and nitric oxide synthases (NOS) (Table 2). These actions on these enzymes contribute to reduce the intracellular ROS formation in EC [47] and to inhibit leukocyte adhesion [48-50].

2.3. Resveratrol decreases the foam cell formation

Under endothelial dysfunction, circulating monocytes adhere to the arterial endothelium, migrate to the subendothelial space, and differentiate into resident macrophages within the subendothelial matrix. OxLDL stimulate the expression of scavenger receptors CD36 and the class A scavenger receptor (SR-A) within monocytes, macrophages and smooth muscle cells (SMC). These receptors internalize the oxLDL in a specific manner, leading to a massive accumulation of cholesterol esters until foam cells are formed. These macrophage-derived foam cells make up the fatty streak that precedes more advanced sclerotic lesions (Figure 6). Resveratrol inhibits the activity and the expression of SMC cyclooxygenase-2 (COX-2) which normally produced prostaglandin E_2 (PGE_2) which up-regulate SR-A expression [51]. Resveratrol could be able to decrease SMC SR-A activity through the decrease of the endothelial growth factor (EGF) and others (IL-1, PDGF, TGFβ) [52]. So, by the reduction of the interaction between oxLDL and macrophage scavenger receptors, resveratrol contributes to prevent early steps in atherogenesis.

2.4. Resveratrol inhibits pro-inflammatory modulators

Resveratrol contributes to reduce the chemokine production which may be responsible for the chemotaxis and accumulation of macrophages in fatty streaks (Figure 6). Resveratrol is able to inhibit interleukin-6 (IL-6) release by stimulated peritoneal macrophages in mice [53, 54], and in cortical mixed glial cells [55]. This action could result from a blocking of calcium ion influx by resveratrol. Moreover, resveratrol contributes to reduce inflammatory response in atherosclerosis when macrophages (or SMC, EC) appear to be activated and produce numerous inflammatory products, such as TNFα, IL-6, monocyte chemoattractant protein-1 (MCP-1).

2.5. Resveratrol inhibits adhesion molecules

Resveratrol inhibits the expression of vascular adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) that mediate the firm adhesion of monocytes to the vascular endothelium in early atherosclerosis stages. Subsequently, resveratrol inhibits monocyte adhesion to human vascular endothelial cells [56, 57].

2.6. Resveratrol inhibits vascular smooth muscle cell proliferation

Resveratrol could delay atherogenesis by inhibition of vascular smooth muscle cell (VSMCs) proliferation [58, 59]. Indeed, resveratrol is able to reduce VSMCs proliferation induced by various mitogens such as serum, endothelin and PGDF. Antimitogenic effects of resveratrol are not mediated by the induction of apoptosis, but appear to be related to blocking G1$\rightarrow$S transition of cell cycle [60, 61] and the DNA synthesis [59]. In fact, resveratrol leads to a reversible arrest in early S phase of the VSMC cycle.

2.7. Resveratrol and the VEGF pathway

Angiogenesis is important in atherosclerosis where endothelial cell migration, proliferation are essential events in this process. Vascular endothelial growth factor (VEGF) co-localizes with endothelial cells, macrophages and SMC in atherosclerotic plaques. Resveratrol inhibits VEGF-induced angiogenesis by abrogating VEGF-mediated tyrosine phosphorylation of vascular-cadherin and its complex partner, β-catenin [62]. The inhibition of VEGF-induced angiogenesis is mediated by the disruption of ROS-dependent Src kinase activation and the subsequent VE-cadherin tyrosine phosphorylation. Resveratrol can also reduce VEGF by its action on NADPH oxidase [48, 49] which regulates the induction of VEGF expression [63] and the VEGF-induced angiogenesis [64].

2.8. Resveratrol and platelet aggregation

Platelets contribute to the rate of development of atherosclerosis and coronary heart diseases through several mechanisms. It has been shown that resveratrol reduces platelet aggregation in human platelet-rich plasma [65-67].

Tableau 2. Resveratrol effects on enzymatic systems implicated in the oxidation of LDLs

Enzyme	Cell systems	Resveratrol actions	Resveratrol concentration	Ref.
NAD(P)H oxidase	Macrophage homogenates	Activity ↘	*Res: 1-100 µM	[49]
	Endothelial cells	Prevents the strain-increased NADPH oxidase activity	Res: 0.1-100 µM	[45]
HX/XO oxidase	Rat cardiac cells	Inhibits XO/xanthine – mediated cytotoxicity	Res: 100 µM	[46]
	Isolated rat leukocytes	Prevents leukocytes recruitment	Res: 0.7 mg/kg	[50]
Myeloperoxidase	Human neutrophils	Activity ↘	Res: 10^{-6}-10^{-2} mg/ml	[68]
	Mouse skin extracts	Normalizes the levels of activity	Res: 1-25 µM	[69, 70]
Superoxide dismutase	Chinese hamster lung	Activity ↗	Res: 100 µg/ml	[71]
	Rat cardiac cells	Activity ↗	Res : 25-100 µM	[46]
Catalase	Chinese hamster lung	Activity ↗	Res: 100 µg/ml	[71]
	Cardiac tissue	Activity ↗	Res: 14 mg/kg/ day	[72]
Glutathione peroxidase	Chinese hamster lung fibroblasts	Activity ↗	Res: 100 µg/ml	[71]
	Human lymphocytes	Activity ↗	Res : 10-100 µM	[73]
Glutathione reductase	Rat cardiac cells	Activity ↗	Res : 25-100 µM	[46]
	Human lymphocytes	Activity ↗	Res : 10-100 µM	[73]
Glutathione-S-transferase	Rat cardiac cells	Activity ↗	Res : 25-100 µM	[46]
	Human lymphocytes	Activity ↗	Res : 10-100 µM	[73]
NQO1	Rat cardiac cells	Activity ↗	Res : 25-100 µM	[46]

Note: *Res: cis-resveratrol; Nicotinamide adenine dinucleotide (NADPH) oxidase; HX/XO: hypoxanthine/xanthine oxidase; NQO1: NAD(P)H:quinone oxidoreductase 1; ↗: increases the activity of the enzyme cited in the first column; ↘: decreases the activity of the enzyme cited in the first column.

3. RESVERATROL AND ANTICANCER ACTIONS

It is essentially based on the results published by Jang *et al.*, in 1997 in the prestigious journal *"Science"* [31] that many scientists are interested in resveratrol actions and the number of papers published has raised dramatically. Indeed, Jang and its colleagues have report the ability of resveratrol to inhibit carcinogenesis at multiple stages (initiation, promotion and tumor progression) (Figure 7) in *in vivo* models of skin tumors [31]. Systemic administration of resveratrol has since been shown to inhibit the initiation and growth of tumors in a wide variety of rodent cancer models (Table 3) (see for review [74, 75]).

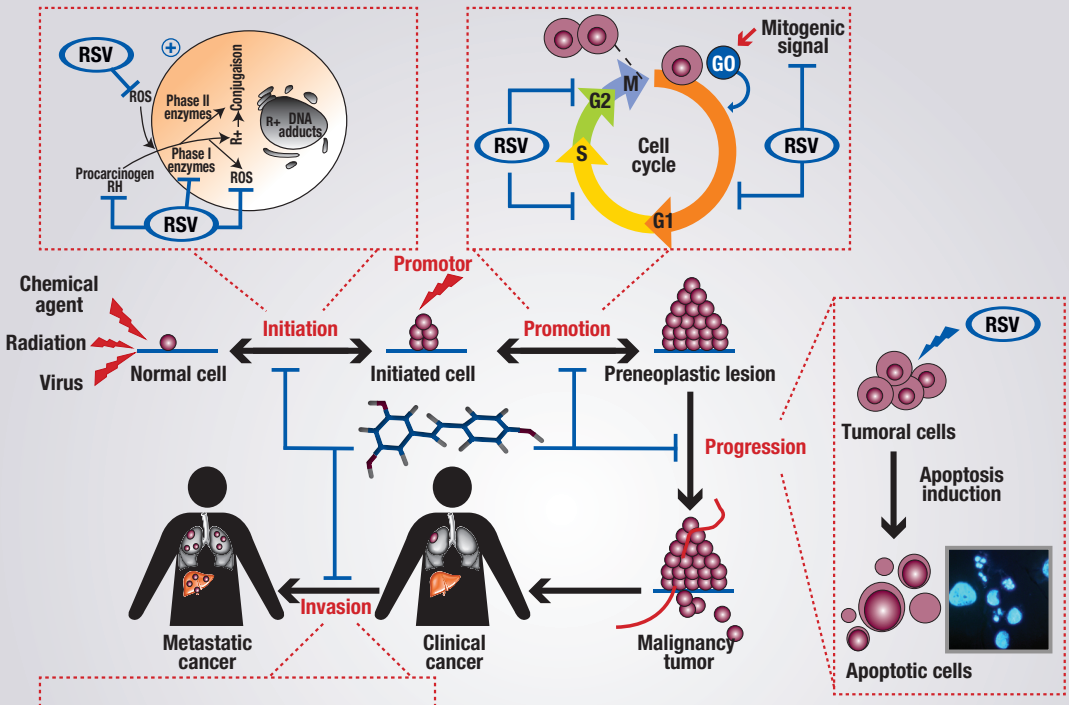


FIGURE 7. **Resveratrol inhibits the different steps of carcinogenesis.** Environmental factors (chemical agents, radiation, virus) can attack normal cells to produce initiated cells. During this first step, resveratrol can block procarcinogens by scavenging reactive oxygen species (ROS), inhibiting the metabolic activation of carcinogens, and/or by the detoxifying increases of phase II enzymes. Then, intiated cells can proliferate during a second steps called promotion. Resveratrol can also block this process through an inhibition of tumoral cell proliferation via an arrest of the cell cycle. In the third steps of carcinogenesis, the tumor progression, resveratrol can induce a programmed cell death named apoptosis of tumoral cells. Finally, the ultimate step is the tumoral invasion, where resveratrol can block this process through an angiogenesis inhibition and the decreases of metastatic dissemination (adapted from Delmas D *et al.*, Curr Drug Target, 2006).

The main properties by which resveratrol is involved in its anticancer activities are:

3.1. Antioxidant properties

Reactive oxygen species (ROS) arise whenever the cell is involved in oxygen utilization, and this production may be exacerbated by xenobiotic drugs. These ROS actively participate in the metabolic activation of procarcinogens and the events associated with the process of carcinogenesis such as oncogene mutation, by modifying the structure of DNA bases. The inhibition of cytochrome P450 by resveratrol can reduce the reactive activation of molecular oxygen. Resveratrol is able to prevent the increase in ROS following exposure to oxidative agents such as tobacco-smoke condensate (TAR), H_2O_2, phorbol esters, ultraviolet radiation [76-78], and to decrease and scavenge ROS [79, 80]. It appears that resveratrol is an effective scavenger of hydroxyl, superoxide, and metal-induced radicals. Resveratrol exhibits also a protective effect against lipid peroxidation in cell membranes and DNA damage caused by ROS [79]. The modulation of antioxidant enzymes could explain the inhibition of resveratrol on DNA damages.

3.2. Antiproliferative properties

Resveratrol is able to inhibit the proliferation of various tumoral cell lines by disturbing the normal progression of the cell cycle. Indeed, various studies report that checkpoint at both G1/S and G2/M of the cell cycle is found to be perturbed by the phytochemicals [81-83]. Checkpoints are controlled by a family of protein kinase complexes, and each complex is composed minimally of a catalytic subunit, cyclin-dependent kinases (cdks), and its essential activating partner, cyclin. Cyclins play a key regulatory role in this process by activating their partner cdks and targeting them to the respective protein

substrates [84]. Complexes-formed in this way are activated at specific intervals during the cell cycle and their inhibition blocks the cell cycle at the corresponding control point. These key regulators can be affected by various stilbenes, especially resveratrol, leading to an arrest of the cell cycle. Interestingly, resveratrol was able to block the different checkpoints of cell cycle in cancerous cell lines-dependent manner. For example, we have shown in hepatocarcinoma and in colon carcinoma cells that resveratrol inhibits proliferation through an arrest of cell cycle in the DNA replication phase, namely S phase, through a modulation of the key regulators of the cell cycle that are cyclins and theirs Cdks [85-88].

3.3. Proapoptotic properties

Induction of apoptosis in precancerous or malignant cells is considered to be a promising strategy for chemopreventive or chemotherapeutic purposes. The induction of apoptosis triggered by polyphenolic compounds has been observed in various cell types with different pathways. Indeed, it has been demonstrated that resveratrol is able to activate cell death by the mitochondrial pathway or by the death receptor pathway. We and others have shown that resveratrol was able to induce apoptosis of various tumor cell lines, especially colon cancer cells through an induction of mitochondrial pathway and through a clustering of death receptors in lipid microdomains called “lipid rafts” to induce the death-inducing signaling complex formation essential to activate caspase pathway [75, 89, 90].

3.4. Antiangiogenic properties

In the same manner as in atherosclerosis, angiogenesis plays an important role in the step of tumoral progression. Indeed, angiogenesis provides a gateway for tumor cells to enter the

circulation and, in the reverse direction, for leukocytes to infiltrate the tumor and provide proteolytic enzymes and chemokines, which facilitate the migration and invasion of tumor cells [91]. This phenomenon occurs through the invasion of endothelial cells from existing vessels in response to multiple extracellular signals such as polyamines, vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF-1, -2). Resveratrol can act on angiogenesis through an inhibition of VEGF, of matrix metalloproteinases, (MMP-9), urokinase-type plasminogen activator and adhesion molecules [52, 92-95].

Table 3. Examples of resveratrol effects in some animal models

Species	Model	Doses	Adminis-tration	Resultat	Ref.
Mice	CCR EG7 cells and CT26 leukaemia cells in s.c.	4 mg/kg une fois	Reg.	Decreases of lymphocytes Treg CD4+/CD25+, TGFβ and increases of IFNγ	[96]
Mice	Prostate cancer cells LNCaP in s.c.	50, 100 mg/kg/j	Reg.	Delays tumor growth and decreases PSA level	[97]
Mice	Leukeamia L1210 cells i.p.	12.5 to 50 mg/kg/j	i.g.	Increases lice survival and the innate (NK) and humoral ; decreases IL-6 production	[98]
Rat	Colorectal carcinogenesis	8 mg/kg/j	*Per os*	Decreases of incidence and tumor volume	[99, 100]
Mice	Melanoma induce by UV	25, 50 µM	Top.	Delays tumor indicence	[101]
Rat	Breast carcinogenesis	12 à 15 mg/kg/j	Reg.	Decreases of tumor number, COX-2, MMP-9 and NFκB activity	[102]
Mice	Spontaneous colorectal carcinogenesis	60 to 240 mg/kg/j	Reg.	Decreases adenoma and PGE_2	[103]

Abd. Abdominal injection, i.g. intragastrique, i.p. intraperitoneal, inj. injection, Reg. Regime, s.c. sous-cutanous Top. Topic

4. RESVERATROL AND IMMUNE SYSTEM / INFLAMMATION

Modulation of immune cells and inflammatory processes represents attractive targets for therapeutic intervention in various diseases such as autoimmune diseases, cancers, chronic inflammation diseases, coronary heart diseases.

Adaptive immune cells (T and B lymphocytes) and innate immune cells (macrophages, myeloid-derived suppressor cells, dendritic cells and natural killer cells) could participate to the immunosurveillance and to the production of proinflammatory cytokines in response to various exogenous stimuli. By its actions on the different immune cells, resveratrol could modulate adaptive and innate immune response. Consequently resveratrol contributes to stimulate immune system in case of infection or to eliminate tumor cells, and then could modulate the inflammatory process [104]. The immune response is limited by the upregulation of anti-inflammatory miRNAs such as miR-146a and miR-146b, which target interleukin-1 receptor associated kinase 1 and tumor necrosis factor receptor-associated factor 6 [105, 106]. Importantly, several reports have recently established a direct link between elevated levels of miR-155 expression and the formation and development of tumors such as leukemia and breast, lung or gastric cancers [107]. Polyphenols are able to modulate the levels of miRNAs which target both oncogenes and tumor-suppressor genes, providing a rational for manipulating the levels of miRNAs depending on the nature and the stage of tumors in order to optimize the anti-tumoral effects of polyphenols. Several downregulated miRNAs by resveratrol such as *miR-17, miR-21, miR-25,* or *miR-92a-2*, are generally considered as onco-miRs [108] and are known to be overexpressed in colorectal cancer [109, 110]. Furthermore, when human THP-1 monocytes were treated with resveratrol before challenging with LPS, it impairs the upregulation of oncogenic pro-inflammatory miR-155 by LPS, at least in part through the upregulation of miR-663, a miRNA targeting JunB and JunD transcripts and regulating TGFβ1 signaling [111].

Furthermore, resveratrol could reduce the production of various cytokines and interleukins produced by immune cells or other disease cells. Moreover, resveratrol can inhibit the lipid mediators such as prostaglandins (PGs) that have been shown to be involved in promoting cell proliferation, suppressing immune surveillance, stimulating tumorigenesis and chronic inflammation [112]. The synthesis of these products from arachidonic acid can occur via to several pathways such as the prostaglandin H synthase (PHS) pathway, the cyclooxygenase (COX), and the lipoxygenase (LOX) pathways (Figure 8). By its action on these key enzymes, resveratrol can block or reduce the inflammatory processes.

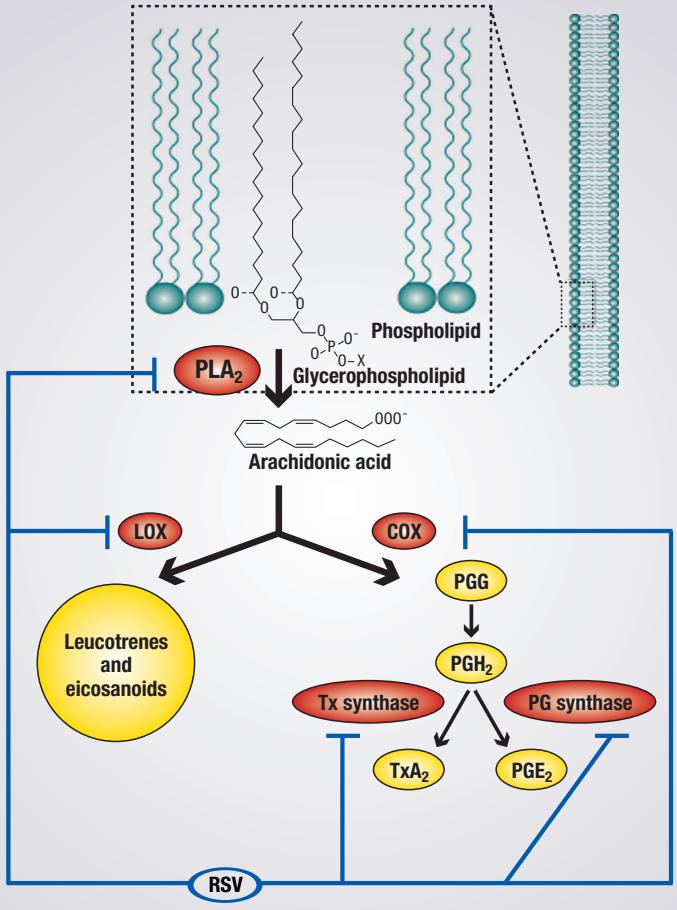


FIGURE 8. **Resveratrol inhibits the different pathways of pro-inflammatory processes** (adapted from Ghiringhelli F *et al.*, Anticancer Med Chem, 2012).

5. RESVERATROL, AGEING AND DEGENERATIVE DISEASES

Aging is the primary risk factor for many diseases including neuro-degenerative diseases [113], cardiovascular diseases [114], stroke [115], diabetes [116], cancer [117], and age-related ocular diseases [118].

Nowadays, the only effective way to slow down the aging process is represented by caloric restriction (CR) [119]. Indeed, the restriction of food intake without malnutrition retards aging or extends the lifespans of yeast, worms, flies, rodents, rhesus monkeys and humans [119-121]. Several evidences support the role of sirtuins, especially sirtuin-1 (SIRT1) activation in the anti-aging effects of CR [122].

SIRT1 is a member of the class III NAD+-dependent histone/protein deacetylases (HDAC). Class III HDACs also known as sirtuins regulate several cellular functions *via* the deacetylation of the acetyl-lysine residues on histones and the transfer of ADP-ribose moiety of NAD+ to acceptor proteins [118]. In particular, SIRT1 has been shown to be involved in the regulation of glucose/lipid metabolism, mitochondrial biogenesis, autophagy, inflammation, circadian rhythms, stress responses, apoptosis and chromatin silencing [123]. Target proteins of SIRT1 include important regulatory proteins and transcription factors such as p53, E2F, NF-κB, FOXO, PGC-1α, HIF and PPAR [123].

The most acclaimed natural activator of SIRT1 is resveratrol [118, 123]. For this reason, it has been proposed that resveratrol could be used to mimic the effect of caloric restriction [124]. Several studies support this hypothesis. Indeed, it has been demonstrated that resveratrol extends the life span of yeast, flies, worms, fish and mice [124]. Moreover, resveratrol modulates insulin secretion, promotes the consumption of glucose by muscle cells, improves insulin sensitivity in peripheral tissues, prevents lipid peroxidation, protects neurons from apoptosis induced by oxidative stress and by inflammation and inhibits the aggregation of β-amyloid peptide [125].

Finally, it's important to note that SIRT1 expression is down-regulated in multiple organs during aging [126] suggesting that resveratrol can be employed to prevent age related-diseases by virtue of its ability to preserve SIRT1 expression [127].

CHAPTER 2

RESVERATROL AND OCULAR DISEASES

Through its benefit health properties, resveratrol could participate in a chemoprevention or in a chemotherapeutic strategy against ocular diseases such as cataract, retinal degeneration, optic neuritis, glaucoma, retinoblastoma and age-related macular degeneration (AMD).

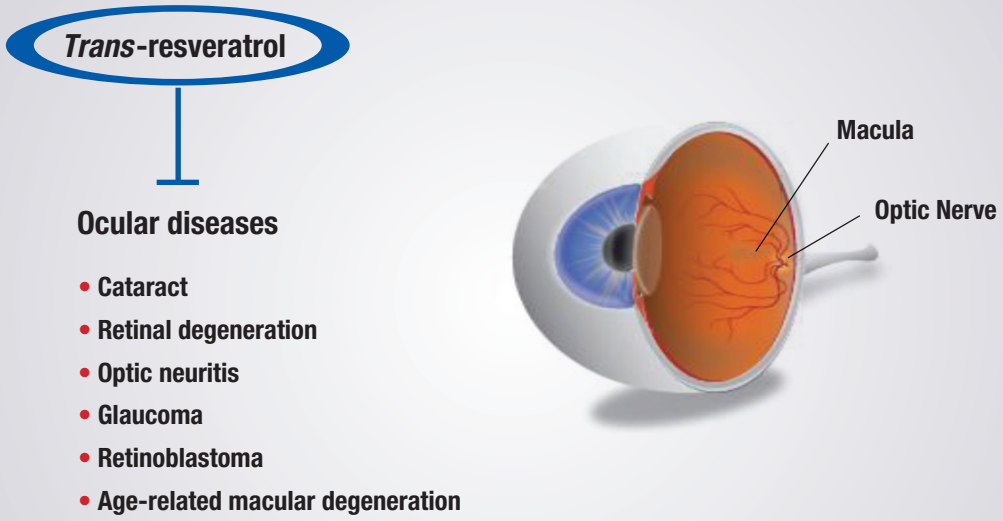


FIGURE 9. Resveratrol could prevent various ocular diseases through its «health benefit» properties.

Through the example of AMD, the following chapter presents the potential effects of resveratrol against this disease.

1. THE AGE-RELATED MACULAR DEGENERATION

Age-related macular degeneration (AMD) is one of the main causes of the deterioration of vision in older adults in developed countries[128]. The results are a loss of vision in the center of the visual field due to damage to the retina. It is usually classed as one of two forms (Figure 10) a dry form characterized by the appearance of drusen which are proteinaceous collections at the level of the retinal pigment epithelium (RPE); and a wet form, in which neovascularisation

complicates the retinal changes. Actually, some therapies are used for wet AMD to inhibit the abnormal growth of blood vessels with VEGF inhibitors or with laser photocoagulation, but some side effects and resistance to these therapies are observed. Concerning dry AMD actually only nutritional supplementation is given, no therapies have shown efficiency. The aim of non exudative AMD treatment is to delay the loss of visual function.

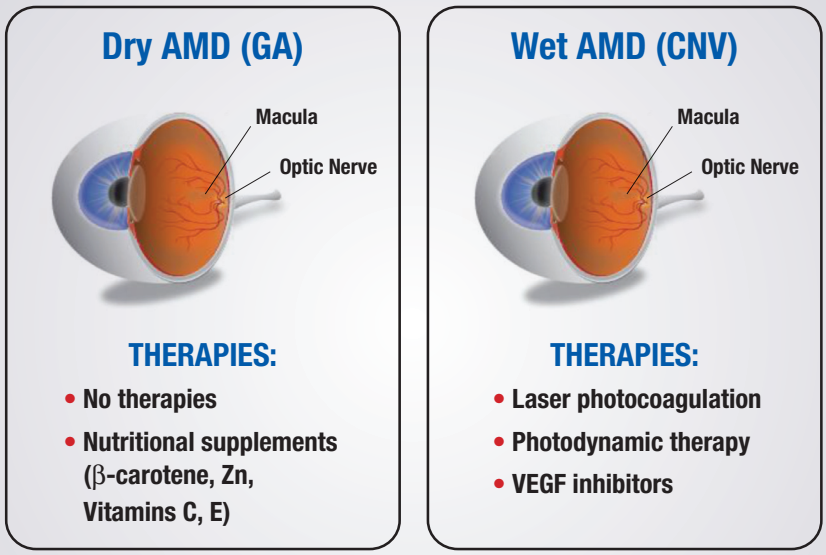


FIGURE 10. **The two forms of AMD**. AMD can present a dry form characterized by the appearance of drusen at the level of the retinal pigment epithelium, and a wet form characterized by a neovascularization. For the first form no therapies are efficiency and for the wet form some therapies are recommended but with some side effects.

Some therapies that modulate risk factors are able to prevent the development or progression of the pathology but do not completely cure patients affected by AMD. Consequently, new therapies are needed and resveratrol could act on this disease at different levels. Indeed, key processes have been identified in AMD such as oxidative damage, impaired activity or function of the retinal pigmented epithelium, increased apoptosis and chronic inflammation.

Moreover, neovascularisation seems to be very important in the AMD complications. RVS could act on these different steps mainly through its antioxidant power, anti-inflammatory action or through its anti-angiogenic actions (Figure 11).

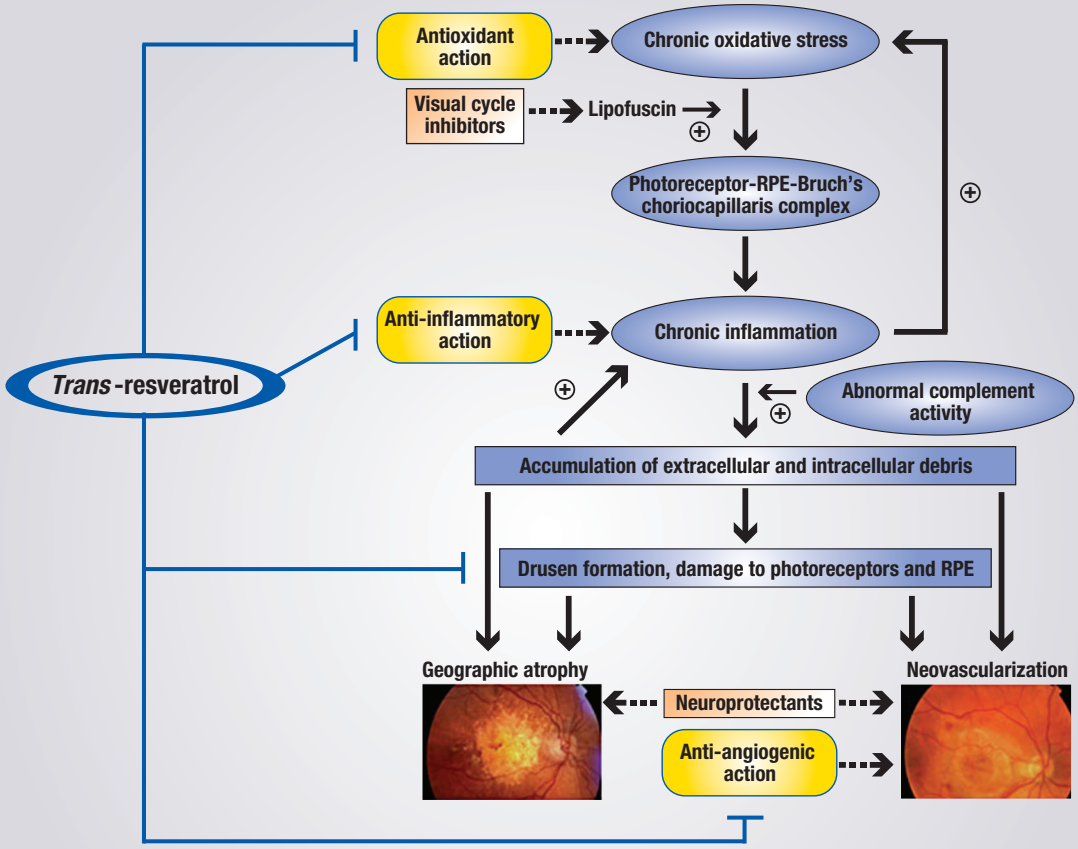


FIGURE 11. **Molecular processes of AMD and the potential effects of resveratrol on the key steps of this disease.** One or more of the following key processes is likely to have a role in AMD, particularly the oxidative stress, the lipofuscin accumulation and impaired function of retinal pigment epithelium (RPE). Subsequently, an increase of cell death is observed as well as an inflammation which is activated by accumulation of extracellular and intracellular debris. Resveratrol could act on the different processes through its 1) antioxydant power; 2) antiinflammatory properties; 3) ability to prevent damage of photoreceptors; 4) antiangiogenic action (adapted from Zhang K *et al.*, Nat Rev Drug Discov, 2012).

2. RESVERATROL AND AMD INITIATION

AMD is a multifactorial disease with both environmental and genetic risk factors [129]: air pollution, smoking, ultraviolet (UV) radiation, metabolic diseases (e.g. diabetes, hypertension, obesity), dietary fat consumption [130-133] and genetic polymorphisms such as *cfh, arms2/hrta1* genes [134]. Alone or combined, these factors could contribute to initiate AMD through the production of free radicals such as superoxide anion ($O2^{\cdot -}$), nitric oxide ($NO^{\cdot}$), hydroxyl radical ($OH^{\cdot}$), that create an oxidative stress and inflammation in ocular tissues (Figure 12). It is now well established that oxidative stress and inflammation play a critical role in the initiation and development of AMD [135]. Due to its antioxidant power and its ability to scavenge free radicals, resveratrol could protect ocular tissues against oxidative stress.

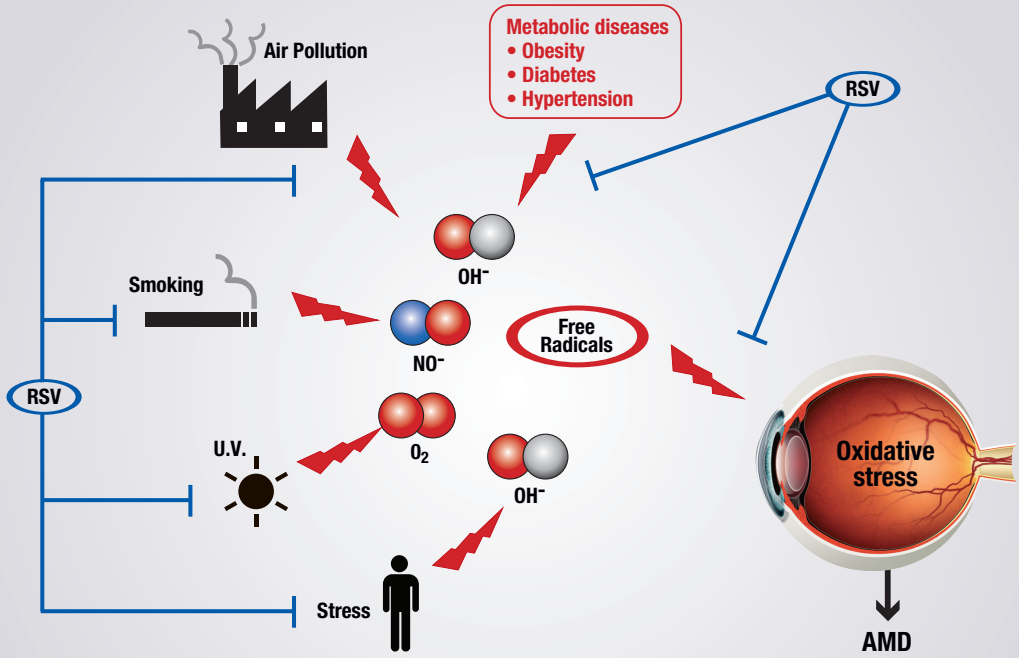


FIGURE 12. **Resveratrol could prevent oxidative stress involved in the development and progression of ocular diseases such as AMD.** Various environmental factors produce free radicals leading an oxidative stress in ocular tissues and consequently provoke the initiation of diseases such as AMD. Resveratrol is able to scavenge free radicals as well as to act on other factors such as metabolic diseases. These Resveratrol properties could prevent or delay ocular diseases links to oxidative stress.

Retinal pigmented epithelium (RPE) cells, which is the a cell layer responsible for maintaining the retina health by providing structural and nutritional support, are the primary target for the AMD-associated oxidative stress [136]. Resveratrol due to its antioxidant power could be efficient in reducing the risk of AMD in a same manner as antioxidant commonly found in fruits and vegetables (vitamins C, E and carotenoids) for which studies have demonstrated that their intake may delay or prevent the development of retinal diseases[137]. Indeed, a treatment of RPE cells with resveratrol prevents the ROS production, both in the basal state (around 20% compared to untreated control) and when cells are treated with an inducer of oxidative stress such as hydrogen peroxide (H_2O_2) (Figure 13). Secondly, resveratrol protects or delays cell death induced by H_2O_2 in RPE cells. Indeed, King *et al*. have shown that a pretreatment of cells with resveratrol followed by a treatment with H_2O_2 prevents cell proliferation inhibition [138] (Figure 13). These protective actions of resveratrol could involve an inhibition of mitogen-activated protein kinase (MAPK) pathways induced by an oxidative stress. At the basal level, resveratrol was able to decrease the phosphorylation of extracellular signal-regulated kinase, phospho-ERK1 and 2 in dose-dependent manner as well as the tyrosine/threonine kinase MEK [138]. This ability to reduce MAPK activation could contribute to reduce the effect of H_2O_2 on this pathway. In fact, a pretreatment of RPE cells with resveratrol followed by H_2O_2, reduced the phosphorylation of ERK 1/2 in dose-dependent manner, especially with 25 and 50 µM resveratrol compared to cells treated with H_2O_2 alone [138].

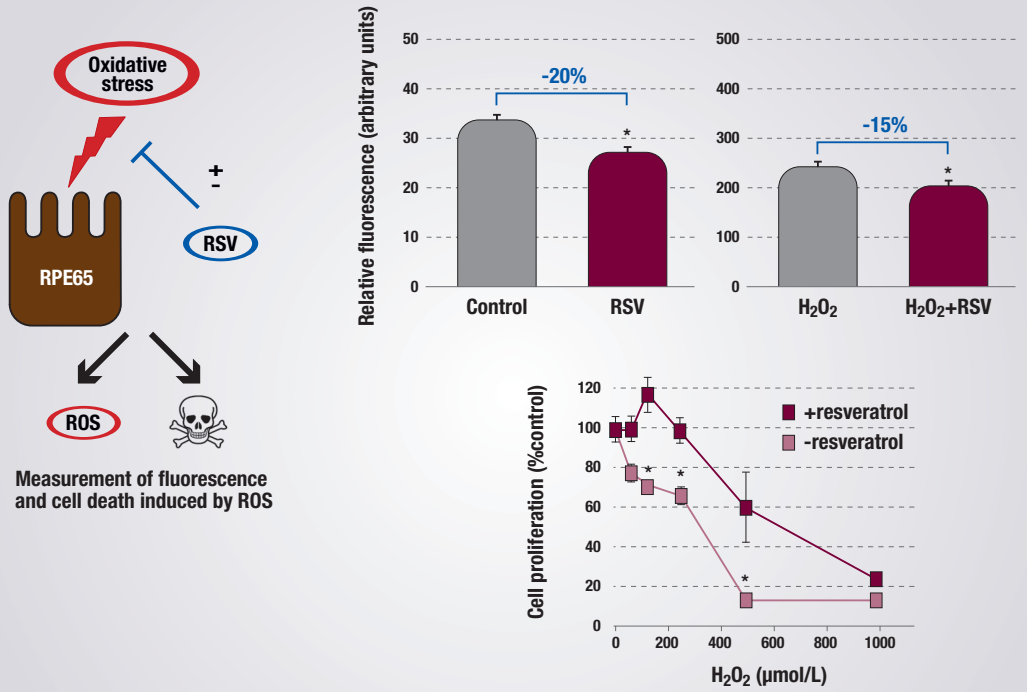


Figure 13. Resveratrol prevents the production of ROS in retinal pigmented epithelium (RPE) cells induced by hydrogen peroxide (A) and prevents the cell death induced by H_2O_2 (adapted from King RE *et al.*, Chem Biol Interact, 2005).

Others environmental factors, such as acrolein found in cigarette smoke induce an oxidative stress in human retinal cells. A study realized in ophthalmology department in Taiwan has shown that resveratrol pretreatment can also prevent damage caused by an exposure to acrolein during seven days followed by H_2O_2 treatment [118, 139].

In similar manner, in our laboratory at Dijon (France), we have shown that toxic effects of oxysterols, that coming from the diet or from cholesterol catabolism, and playing important roles in AMD could be counteracted by resveratrol [140]. Indeed, when retinal cells (ARPE) are treated with 7-beta-hydroxycholesterol (7β-OH) or with 7-ketocholesterol (7KC), we observed a decrease of the viable cells after 24h and more strongly after 40h (Figure 14). Resveratrol, which has any toxic effect on retinal cells, can protect them from the toxic effects of oxysterols (Figure 14).

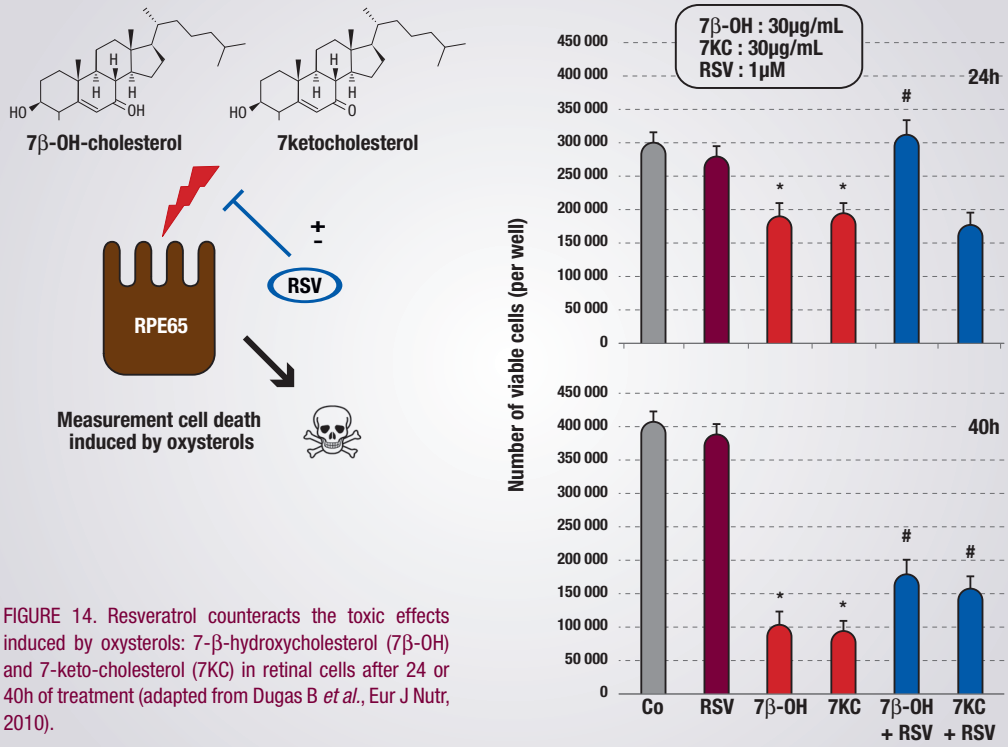


FIGURE 14. Resveratrol counteracts the toxic effects induced by oxysterols: 7-β-hydroxycholesterol (7β-OH) and 7-keto-cholesterol (7KC) in retinal cells after 24 or 40h of treatment (adapted from Dugas B *et al.*, Eur J Nutr, 2010).

Other antioxidant mechanisms of resveratrol could be involved. Indeed, we have shown in various cell lines that resveratrol could act on the ROS production via its action on mitochondrial enzymatic pathways [75].

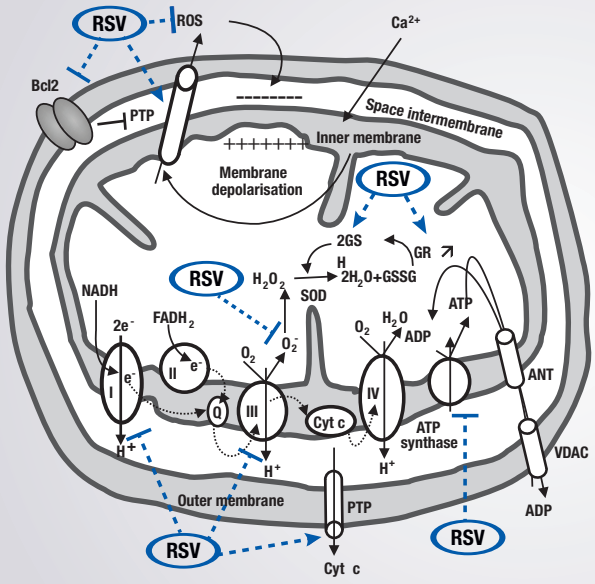


FIGURE 15. Resveratrol counteracts the production of free radicals from mitochondria through a modulation of various enzymatic pathways such as glutathione (GSH) levels, glutathione peroxidase (GPX), glutathione reductase (GR), glutathione-S-transferase (GST), and catalase (CAT) activities. Resveratrol accelerates detoxification of anion superoxide and scavenges this anion (adapted from Delmas D *et al.*, Anticancer Agent Med Chem, 2011).

Resveratrol can interfere with mitochondrial electron transport and favors a fall in the mitochondrial transmembrane potential $\Delta\psi_m$ [141, 142], a decrease in ATP production and the generation of ROS [143]. Resveratrol has been also described 1°) decrease complex III activity by competition with coenzyme Q, which complex is the site of ROS production, 2°) inhibit ATPase activity and 3°) scavenge the superoxide anion (Figure 15) [144]. Furthermore, $O_2^{\cdot-}$ radicals are converted to H_2O_2 by superoxide dismutase (SOD), and H_2O_2 detoxification normally occurs according two different reactions, either by the thioredoxine reductase (TR) or by reacting with glutathione reductase (GSH) by glutathione peroxidase (GPO). The latter reaction produces water and oxidized glutathione (GSSG), and GSSG is recycled to GSH by glutathione reductase. So, on top of scavenger $O_2^{\cdot-}$, resveratrol accelerates the detoxification of $O_2^{\cdot-}$ by inducing an increase in glutathione (GSH) levels and also by inducing glutathione peroxidase (GPX), glutathione reductase (GR), glutathione-S-transferase (GST), and catalase (CAT) activities [73, 145-148] and their mRNAs levels (Figure 15) [149]. This modulation of antioxidant enzymes could explain the inhibition of DNA damage in human lymphocytes induced by various toxic drugs (i.e. hydrogen peroxide, 1,2-dimethylhydrazine, bleomycin) [99, 150, 151].

Metabolic diseases can play also an essential role in the production of oxidative stress. It is well now defined that diabetes, obesity have many impacts on ocular diseases [152]. A wide range of evidences implies that dietary hyperglycemia is etiologically related to human aging and diseases, including diabetic retinopathy and AMD. In this context, these diseases can be considered as metabolic retinal diseases. Some clinical trials have been published examining the effects of resveratrol on whole-body energy metabolism in relation to the multiple health factors that are affected by obesity and type 2 diabetes [153-157]. Indeed, a daily administration of 2.5 or 5 g resveratrol during 28 day shows a decrease of fasting and

postprandial glucose and insulin. At low concentration such as 5 mg twice daily for 4 weeks, resveratrol significantly decreased insulin resistance [158]. In a healthy obese men, Timmers *et al*., have shown 75 mg of resveratrol twice daily for 30 days improved the metabolic profile: resveratrol reduced sleeping and resting metabolic rate [155]. In muscle, resveratrol activated the AMPK–SIRT1–PGC1α axis, reduced blood glucose and insulin levels, reduced liver fat, improved muscle mitochondrial function, and reduced inflammation marker in the blood [155]. Consequently through the pleoitropic action of resveratrol on metabolic diseases, this polyphenol could contribute to reduce the collateral effects of metabolic diseases on eye disorders especially on AMD.

A last important mechanism in this step of AMD initiation is the accumulation of lipofuscin or cellular debris in RPE. Lipofuscin, an ageing pigment, is considered as a creditable marker for the ageing of cells. Lipofuscin tends to accumulate even at an early age, but rapidly progresses with the advancement of ageing process suggesting the inability of autophagy to handle the garbage disposal capacity. Decline of autophagy during ageing appears to be the cause for lipofuscin accumulation. Morselli *et al.* have shown that transgenic expression of SIRT1 induces autophagy in human cells *in vitro* and in C. elegans *in vivo* [159] Caloric restriction and resveratrol promotes longevity through the SIRT1-dependent induction of autophagy [160] (Figure 16).

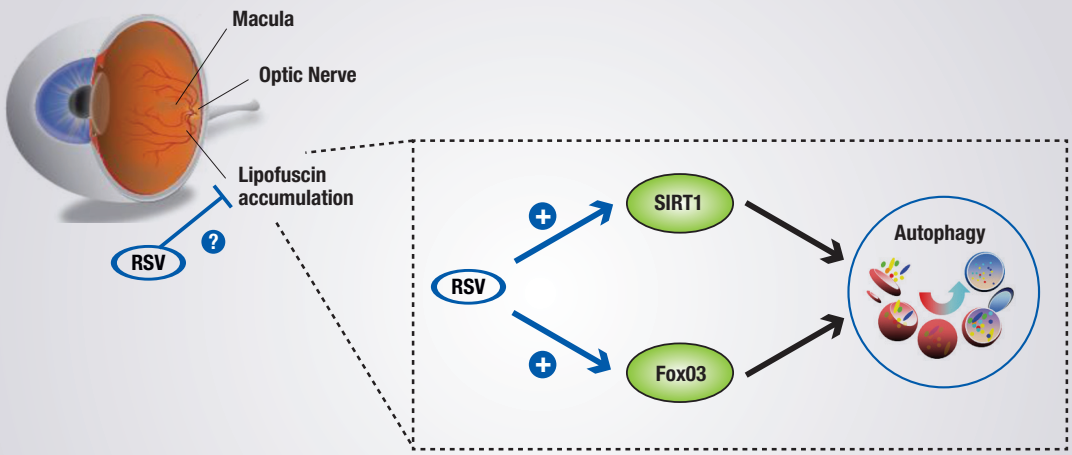


FIGURE 16. Resveratrol could reduce lipofuscin accumulation in RPE through an increase of autophagy process *via* an activation of SIRT1 and Fox03 (adapted Gurusamy et *al.*, Cardiovasc Res, 2010).

3. RESVERATROL AND INFLAMMATORY PROCESSES RELATED TO AMD

In the previous chapter, we have shown that resveratrol is an efficiency anti-inflammatory compound in various models of cardiovascular diseases, cancer or chronic inflammatory diseases. Ocular diseases involve also inflammatory processes especially in AMD [161-163] and then resveratrol could reduce them in AMD.

Based on the important role played by interleukins, a study has shown that resveratrol was able to reduce the interleukins IL-6 and IL-8 production induced by the glucose in retinal cells (Figure 17) [164]. This last interleukin IL-8 has been shown an important risk factor for AMD [163].

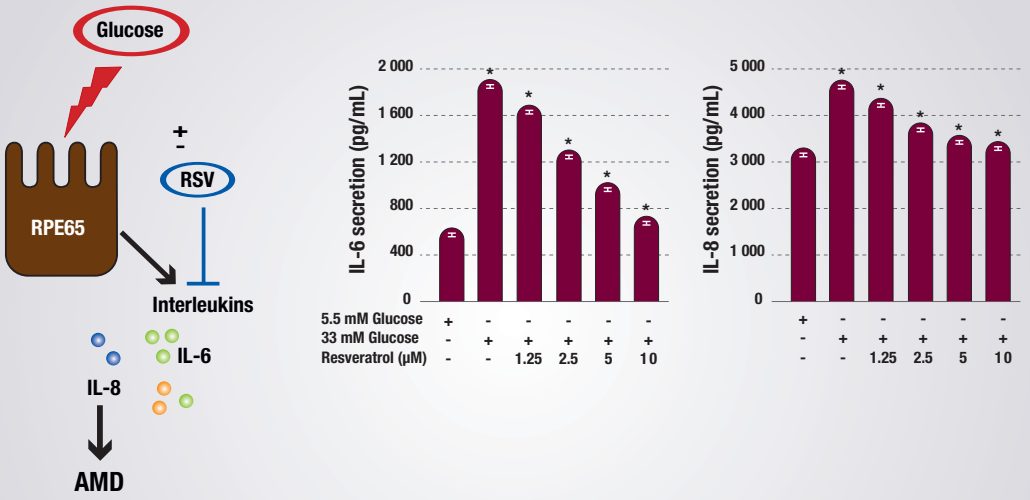


FIGURE 17. Resveratrol prevents interleukins secretion and consequently prevents inflammatory effects induced by glucose in retinal cells (adapted Losso JN *et al.*, J Agric Food Chem, 2010).

4. RESVERATROL AND AMD COMPLICATIONS

The ultimate steps of AMD process is the neovascularisation which constitute a major complication of AMD. The molecular mechanisms involve the vascular endothelial growth factor A (VEGFA) and an increase of vascular permeability that results in loss of vision [165]. Antiangiogenic therapies targeting VEGF have proven to be highly effective in treating neovascular AMD, but they present some side effects.

In this way of angiogenesis inhibition, resveratrol could counteract AMD through its action on the VEGF contributing to the abnormal growth of blood vessels. To test this hypothesis, we have tested the protective effect of resveratrol on the VEGF secretion induced by oxysterols in RPE cells [140]. Oxysterols induce a VEGF secretion after 24h and 40h of treatment with the 7βOH and the 25-hydroxycholesterol, (25OH) (Figure 18). Interestingly, a cotreatment with resveratrol at 1 µM decreases VEGF secretion induced by these oxysterols both at 24h and 40h [140].

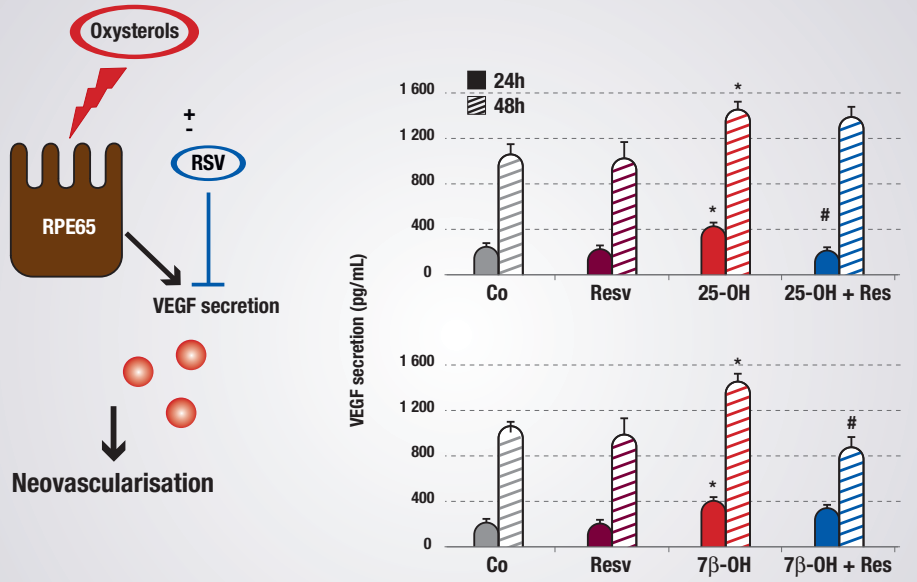


FIGURE 18. Resveratrol prevents VEGF secretion induced by oxysterols (25-hydroxy-cholesterol, 25OH; 7βhydroxy-cholesterol, 7β OH) in RPE cells after 24 or 40 h of treatment (adapted from Dugas B *et al.*, Eur J Nutr, 2010).

Consequently resveratrol could reduce neovascularisation or protect against various factors that induce VEGF production and promote neovascularisation such as diabetes which is known to induce VEGF and which is linked to the progression of vision loss. In this optic, Kim *et al.*, have shown that resveratrol pretreatment can prevent VEGF increase by diabetes in retinal tissues [166]. The authors of this study show an increase of VEGF observed between the outer plexiform layer and the nerve fibre layer on retinal sections of mice, two months after induction of diabetes, comparatively to the control mice (Figure 19). A resveratrol pretreatment of mice by oral gavage at 20 mg/kg once a day for 4 weeks prevents VEGF secretion as well as VEGF expression (Figure 19).

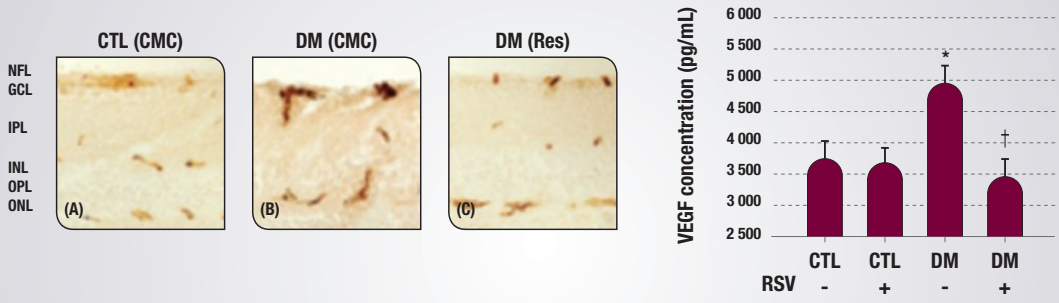
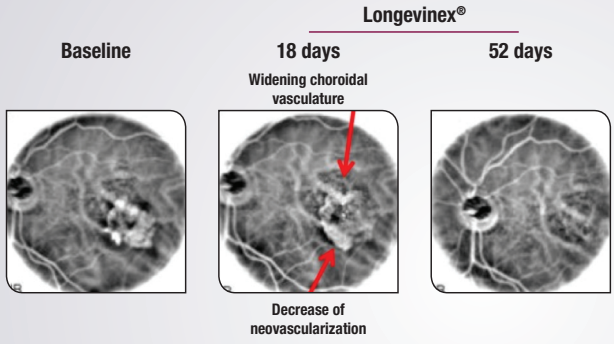


FIGURE 19. **Diabetes-induced VEGF levels reduced by resveratrol.** Mice were intraperitoneally injected with 55 mg/ kg of streptozotocin for 5 consecutive days to induces diabetes (DM). An increase of VEGF is observed between the outer plexiform layer (OPL) and the nerve fibre layer (NFL), in brown color comparatively to the control mice receiving only vehicle. VEGF increased by diabetes was prevent by resveratrol pretreatment (oral gavage at 20 mg/kg once a day for 4 weeks, beginning 1 month after the fifth injection of streptozoticin). A molecular analysis revealed that diabetes increase VEGF concentration was prevent by resveratrol pretreatment (adapted from Kim YH *et al.*, Acta Ophtal, 2012).

Recently, a clinical trial realized in the USA in octogenarians have shown that an oral administration of Longevinex® which is a combination of resveratrol with quercetin, ferulic acid along with vitamin D3 and a cooper/ iron/calcium binding molecule called IP6 (inositol hexaphosphate) could reduce neovascularisation (Figure 20) [167].



Longevinex® composition:
- **resveratrol (100 mg per capsule),**
- **quercetin,**
- **ferulic acid/Vit D3 (1.200 IU),**
- **IP6.**

FIGURE 20. **Longevinex with resveratrol content reduces neovascularization after 52 days of treatment in humans.** 75 y/o vietnam veteran with prolonged post-traumatic stress disorder, diabetes and dry AMD × 10 years who developed wet AMD in his left eye six months ago but adamantly refused repeated requests to do invasive diagnostic (fluorescein angiography) and intravitreal Lucentis® injections or any other type of injection(s). He started Longevinex® and reported better vision in five days and passed his driver's license after seven capsules. At his two week clinical appointment, the authors see objective retinal and visual restoration, similar to anti-VEGF therapy (adapted from Richer S *et al.*, Nutrients, 2013).

5. RESVERATROL AND EYE AGING

Resveratrol could also act by other mechanisms particularly through sirtuins that are recently described as essential target in various process such as cellular stress resistance, genomic stability, tumorigenesis and energy metabolism [168]. The sirtuin-1 desacetylase (SIRT1) is found in various organs and tissues regulating various pathways such as glucose production in liver, fat mobilization and lipid metabolism in adipose tissue or also angiogenesis in blood vessel or other process in brain, pancreas or intestine. SIRT1 is expressed in almost all of the ocular tissues, including the cornea, lens (epithelial cells), iris, ciliary body, and retina (Figure 21A). Particularly, in the cornea, SIRT1 is localized in the corneal epithelial cells, keratocytes, and corneal endothelial cells. In the retina, SIRT1 is found in the retinal pigment epithelium (RPE), outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL).

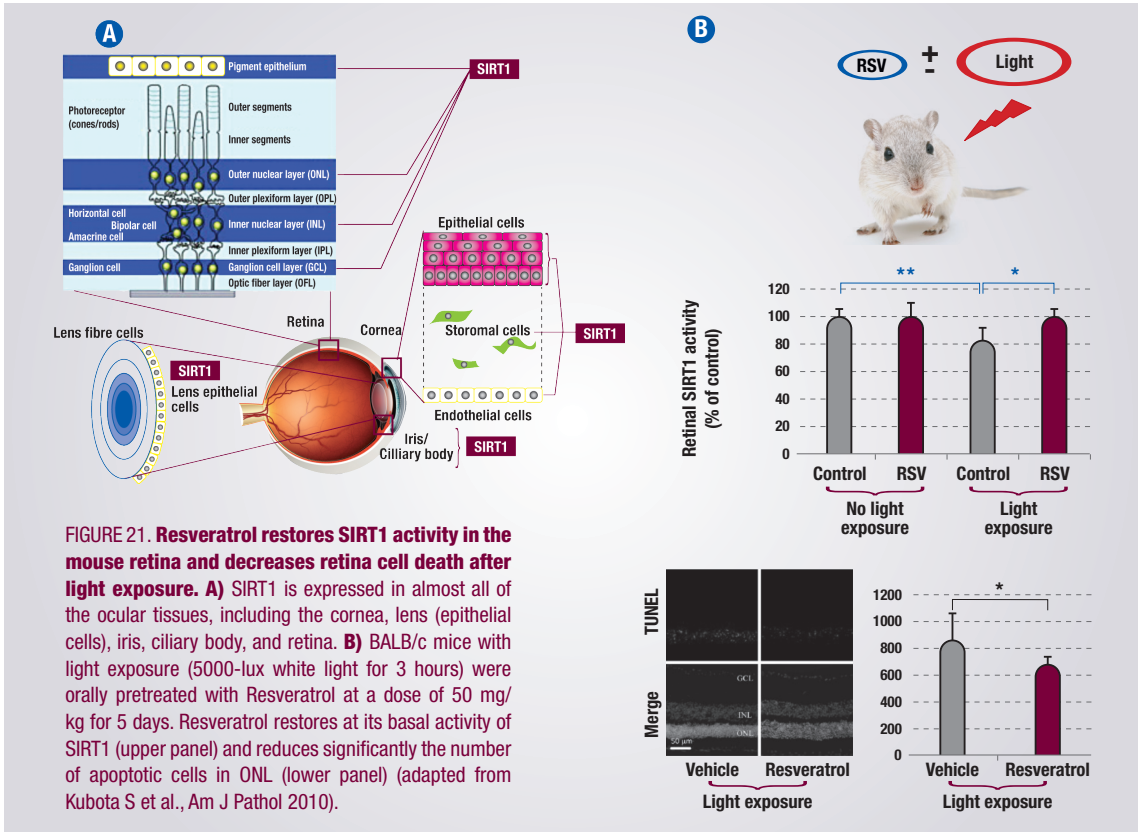


FIGURE 21. **Resveratrol restores SIRT1 activity in the mouse retina and decreases retina cell death after light exposure. A)** SIRT1 is expressed in almost all of the ocular tissues, including the cornea, lens (epithelial cells), iris, ciliary body, and retina. **B)** BALB/c mice with light exposure (5000-lux white light for 3 hours) were orally pretreated with Resveratrol at a dose of 50 mg/kg for 5 days. Resveratrol restores at its basal activity of SIRT1 (upper panel) and reduces significantly the number of apoptotic cells in ONL (lower panel) (adapted from Kubota S et al., Am J Pathol 2010).

In eyes, SIRT1 seems to protect the retinal cells from DNA damage such as oxidative stress-related retinal damage, apoptotic retinal death and anti-inflammation. On the other hand, the breakdown of SIRT1 causes retinal damage through multiple mechanisms. Thus, resveratrol which is described as an agonist of sirtuins could protect eye by SIRT1 activation. In this way, Kubota *et al*., have shown that oral resveratrol pretreatment at a dose of 50 mg/kg for 5 days reverses retinal SIRT1 activity to restore at its basal activity in BALB/c mice exposed to 5000-lux white light for 3 hours (Figure 21B) [169]. These events are associated with a significantly decreases of the number of apoptotic cells in the outer nuclear layer (ONL) (Figure 21B).

CONCLUSION AND PERSPECTIVES

They are compelling evidences that resveratrol could act on various pathologies such as coronary heart damages, cancers, degenerative diseases by affecting various pathways. Among these pathways, resveratrol is able to act on common targets such as reactive oxygen species, lipid mediators, apoptosis, proinflammatory mediators and angiogenesis. Through these mechanisms, resveratrol could prevent age-related ocular diseases, especially AMD and could protect eyes against environmental factors (such as diabetes, hypertension, stress, UV light, acrolein, found in cigarette smoke, air pollution). Moreover, resveratrol does not present cytotoxicity in animal models and in normal cells at the concentrations usually used in vitro (up to 100 µM) or in vivo (up to 500 mg/kg/day; see table 3 [170-173]. Moreover some clinical studies have shown that resveratrol is safe in human with various doses. Indeed, after resveratrol administration (0.5, 1.0, 2.5 or 5.0 g) daily for 29 days in forty healthy volunteers, no significant adverse effects were observed, only mild to moderate gastrointestinal symptoms with 2.5 and 5.0 g of resveratrol [174]. Other reports on trials of resveratrol in humans after single [175, 176] or multiple daily doses of up to 600 mg/d administered over 2 or 3 days [177, 178], show that resveratrol is safe under the tested conditions.

Recent clinical studies have shown that patients with confirmed colorectal cancer present high level of resveratrol metabolites, especially resveratrol-3-O-sulphate, resveratrol-3-O-glucuronide and resveratrol-4-O-glucuronide, accumulated in the colorectum and we have recently shown that these metabolites present anticarcinogenic properties. The question can then be asked whether resveratrol or resveratrol metabolites could be accumulated in eyes and ocular tissues and if it is the aglycone molecule or its metabolites that are active?

Finally, through its properties, resveratrol could constitute a good candidate to prevent ocular diseases, and additional studies are now required to address the mechanisms by which resveratrol could provide a novel strategy to enhance the efficacy of therapy used actually.

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