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RESVERATROL AS PROMISING NATURAL RADIOPROTECTOR. A REVIEW

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ABSTRACT

Public feelings concerning radiation are still controversy. The main sources of trouble seems to be the failure nuclear power plant and danger of terroristic attack, which may cause temporally enhanced level of radiation leading to harmful health effects. Since radiation induced cellular damage is attributed primarily to harmful effect of free radicals, molecules with direct free radical scavenging properties are particularly promising as radiation modifiers/protectors, i.e. agents which present prior to or shortly after radiation exposure alter to response of tissues to radiation. Unfortunately, some of known radioprotectors are toxic at doses required for radioprotection.

Resveratrol (RSV), an natural polyphenol is produced in several plants in response to injury, stress, bacteria or fungi infection, UV-irradiation and exposure to ozone. It is present in human diet i.e. in fruits and in wine. RSV is known for its antioxidant, anti-inflammatory, analgesic, antiviral, cardioprotective, neuroprotective and antiageing action and it has been shown to have chemopreventive effects with respect to several human disease such as cardiovascular disease, osteoporosis and gastric ulcers. Depending on the dose, RSV may act as antioxidant or as pro-oxidant. RSV improves sperm count and motility in rodents and prevent DNA damage caused by cryptopreservation of human sperm. Moreover, RSV acting with other agents, inhibits the toxic action of them. There are evidences that RSV is able to modulate the behavior of cells in response to radiation induced damage.

Minimalization of radiation induced damage to somatic and germ cells by RSV might be useful in cancer therapy to prevent the damage to normal cells as well as in case of radiological accidents.

Key words: *resveratrol, irradiation, radioprotection*

STRESZCZENIE

Odczucia społeczne związane z oddziaływaniem promieniowania jonizującego są kontrowersyjne. Głównym źródłem obaw są zagrożenia związane z możliwością awarii elektrowni atomowych oraz z groźbą zamachów terrorystycznych, które mogą spowodować czasowe podniesienie poziomu promieniowania na określonym terenie prowadząc do wystąpienia niekorzystnych skutków zdrowotnych. Z uwagi na to, że uszkodzenia indukowane w komórkach przez promieniowanie jonizujące są spowodowane głównie przez wolne rodniki, substancje nietoksyczne posiadające zdolność ich neutralizowania są pożądane jako modyfikatory działania promieniowania lub związki chroniące tkanki przed działaniem promieniowania. Niestety, używane dotychczas radioprotektory są najczęściej toksyczne w dawkach wymaganych dla celów ochrony radiologicznej.

Resweratrol (RSV), polifenol pochodzenia naturalnego, wytwarzany jest przez niektóre gatunki roślin w odpowiedzi na uszkodzenie mechaniczne, stres, infekcję bakteryjna lub grzybiczą, działanie promieniowania UV lub ozonu. Wchodzi w skład diety człowieka, obecny jest m.in. w owocach i winie. RSV posiada właściwości antyoksydacyjne, przeciwzapalne, przeciwbólowe, antywirusowe, kardioprotekcyjne, neuroprotekcyjne oraz przeciwdziała starzeniu się, w związku z tym zapobiega różnym schorzeniom takim jak choroby układu krążenia, osteoporoza, owrzodzenie żołądka. Resweratrol może wykazywać działanie antyoksydacyje lub prooksydacyjne w zależności od wielkości zastosowanej dawki. Wydaje się być pomocny w leczeniu niepłodności męskiej. Powoduje zwiększenie liczebności i ruchliwości plemników gryzoni oraz przeciwdziała uszkodzeniom DNA powstałym podczas przechowywania ludzkiego nasienia. Ponadto, RSV podawany jednocześnie z innymi

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czynnikami przeciwdziała ich toksycznemu działaniu. Znane są przypadki niwelowania przez resweratrol niekorzystnego działania promieniowania.

Minimalizacja uszkodzeń indukowanych w komórkach somatycznych i rozrodczych człowieka przez resweratrol mogłoby znaleźć szerokie zastosowanie, szczególnie w odniesieniu do ochrony zdrowia osób pracujących przy usuwaniu skutków awarii i wypadków radiacyjnych oraz dla ochrony zdrowych tkanek pacjentów poddawanych radioterapii nowotworowej.

Słowa kluczowe: *resweratrol, promieniowanie, ochrona przed promieniowaniem*

INTRODUCTION

All living organisms including human are continuously exposed to naturally and man-made sources of ionizing radiation. There are a number of occupation in which employees are exposed to man-made sources of radiation, such as medical personnel, uranium miners, nuclear plant workers and other employees using radiation for industrial and scientific purposes. Ionizing radiation may be emitted in the process of natural decay of same instable nuclei or following excitation of atoms and their nuclei in nuclear reactions, cyclotrons, X-rays machines and others instruments. The natural source of ionizing radiation are cosmic rays, terrestrial radionuclide's that occur in the Earth's crust, in building materials and in air, water and food, and in the human body itself. The greatest man-made source of human exposure is radiodiagnosis and radiotherapy. Controlled exposure to ionizing radiation is one of the most used treatments of cancer patients [83].

Public feelings concerning radiation are still controversy. On the one hand the people understand advantages, coming from radiation, especially for health, but on the other hand they afraid of radiation. The main sources of trouble seems to be the failure nuclear power plant and danger of terroristic attack, which may cause temporally enhanced level of radiation leading to harmful health effects.

Ionizing radiation represents electromagnetic waves, and particles that can ionize, i.e. remove an electron from an atom or molecule of the medium through which they propagate. Ionization provoked by radiation begins many chemical reactions leading to serious changes in atoms and molecules. The process of ionization changes atoms and molecules and may thus damage cells. There are two primarily mechanisms of interaction of ionizing radiation with biological matter, direct effects, owing to deposition of energy with a macromolecule, and indirect effects, the interaction of energy with water to produce reactive oxygen species (ROS). For X-rays and γ-rays 60 % of damage is connect by indirect effects [6].

One of the most important effect of ionizing radiation in the organism is disorders in synthesis of DNA. Other key effect is induction of DNA damage usually affecting the metabolism, cell-cycle arrest or causing cell death, and possible leading in consequence, depending on the total dose, dose rate and species, to mutagenesis and carcinogenesis [30, 45, 75, 77]. Eukaryote organisms have evolved to develop effective molecular mechanism such as DNA damage response, to detect DNA lesions, signal their presence and promote their repair [66]. Thus, radiation induced damage may be reversible, but in some cases the repair is inaccurate [57, 64] resulting in acute adverse effects within hours to weeks or delayed effects within months to years after exposure. Although DNA damage can cause cell death and eliminate potentially dangerous cells, miss-repaired damage may result in chromosomal damage or mutations. The resulting modification will be transmitted to further cells and may eventually lead to cancer. If DNA damage leads to germ cells mutation, they may be passed to the progeny of the irradiated person and onward through future generation. Consequently, DNA is considered the major target of ionizing radiation damage.

As has been mentioned before approximately 60 % of damage caused by ionizing radiation is connected to production of reactive oxygen species (ROS), such as superoxide and hydroxyl radicals. Since radiation induced cellular damage is attributed primarily to harmful effect of free radicals, molecules with direct free radical scavenging properties are particularly promising as radiation modifiers/protectors, i.e. agents which present prior to or shortly after radiation exposure alter to response of tissues to radiation. Similarly, agents which may be used to minimalize toxicity even applied after radiation are usually called mitigators [17]. Unfortunately, some of known radioprotectors such as the sulphydryl compounds cysteine and cysteamine are toxic at doses required for radioprotection [84]. There are chemicals e.g. endocrine disruptor bisphenol A, which after combined action with ionizing radiation showed different effects depending on tissue, assay and time [28, 38]. On the other hand there are chemicals, which co-administered with ionizing radiation, even at low doses, may enhance the effects induced by each agent alone [26, 27]. So, the finding of non-toxic and radioprotective or/and radiomitigative agent seems to be very useful to health prevention.

CHARACTERISTIC AND OCCURRENCE OF RESVERATROL

Resveratrol (RSV), a white powder with slight yellow cast, is a stilbenoid, an natural polyphenol structurally similar to diethylstilbestrol and estradiol. The molecular formula of RSV is $C_{14}H_{12}O_3$ and the general name is 3,5,4'-thihydroxystilbene, however sometimes there are used alternatively also other names: 3,4',5-stilbenetriol, (*E*)-5-(*p*-hydroxystyryl)resorcinol, (*E*)-5-(4-hydroxystyryl)benzene-1,3-diol. RSV exists naturally as both *cis*- and *trans*- isomers. Due to lack of stability and no commercial availability of *cis*-isomer as well as greater natural presence and higher biological activity of *trans*-isomer, the most researchers have used *trans*-isomer is their studies [86].

RSV was first identified as the principal active ingredient from the dried roots of *Polygonum cuspidatum*, used in Japanese and Chinese traditional medicine [59]. RSV is produced in several plants in response to injury, stress, bacteria or fungi infection, UV-irradiation and exposure to ozone [8, 36, 73]. RSV is present in human diet i.e. in fruits such as grapes, peanuts, strawberry, blueberry, cranberry, mulberry, lingberry, sparkleberry, bilberry and in flowers and leaves such as butterfly orchid tree, eucalyptus, spruce, lily, gnetum etc. [49, 56, 68]. RSV is also present in wine, especially in red. Therefore, the skin of red grapes and red wine are considered as a major source of resveratrol in food. The most frequently, RSV is consumed in Mediterranean diet in form of peanuts, grapes and wine. Since RSV is present in wine, it has been postulated that it might be the reason for the "French Paradox", the phenomenon in which the French population has significantly lower incidence of cardiovascular diseases in spite of consumption high-fat diet [85].

BENEFICIAL HEALTH EFFECTS OF RESVERATROL

Numerous data are available on the action of resveratrol. It is currently recognized as bioactive molecule with potential beneficial effects on health due to its pharmacological properties, and lack of harmful effects [7, 47-48]. RSV also is famous because of its substantial commercial applications. RSV is involved in the modulation of several biological processes including the regulation of carcinogenesis. It inhibits the growth of cell lines delivered from various human cancers [69].

RSV is known for its antioxidant, anti-inflammatory, analgesic, antiviral, cardioprotective, neuroprotective and antiageing action [7, 10, 36, 50, 62]. It has been shown to have chemopreventive effects with respect to several human disease such as cardiovascular disease, osteoporosis and gastric ulcers [19, 21, 25, 29]. RSV inhibits apoptotic cell death, thereby providing protection from various diseases including myocardial ischemic reperfusion injury, atherosclerosis, ventricular arrhythmias and cerebral ischemic [29, 87]. Moreover, RSV has been suggested to have ability to protect DNA as well as to induce DNA repair [16].

The effect of RSV on health benefits depends on dose. At lower doses RSV can be very useful in maintaining the mammalian including human health, whereas at higher doses it has pro-apoptotic actions on healthy cells and kill tumor cells. Lower doses of RSV acts as an antiapoptotic agent, providing cardioprotection by increased expression in cell survival proteins, improved post-ischemic ventricular recovery and reduction of myocardial infarct size and cardiomyocyte apoptosis and maintains a stable redox environment. At higher doses, RSV act as a pro-apoptotic compounds, inducting apoptosis in cancer cells by exerting a death signal. At higher doses it depresses cardiac function, elevates levels of apoptotic cells protein expressions, results in an unstable redox environment, increases myocardial infarct size and number of apoptotic cells. At high dose not only hinders tumor growth but also inhibits the synthesis of RNA, DNA and proteins, causes structural chromosome aberrations, chromatin breaks and exchanges, weak aneuploidy, higher S-phase arrest, blocks cell proliferation, decreases wound healing, endothelial and vascular cell growth factor and angiogenesis in healthy tissue cells leading to cell death [59]. For example, resveratrol at dose of 160 μM or higher decreased cell survival in human glioma U87 cells, whereas no toxic effects was observed at 80 μM of RSV [53]. Low dose of RSV improve cell survival as in cardio- and neuroprotection, whereas high doses increase cell death as in cancer treatment [12].

Also, in the animal study, has been stated that the effects of RSV depends on the dose. *Juan* et al. [48] observed no harmful effects as assessed by growth, haematology, clinical chemistry and histopathology in male rats exposed to 20 mg/kg RSV for 28 days. Similarly, the 90-days administration 20 mg/kg of resveratrol daily did not cause harm to the rats [46]. Contrary, doses 1000-3000 mg/kg daily cause damage in rodents kidney and lead to die within 3-4 months [20, 61].

RESVERATROL AS FREE RADICAL SCAVENGER

Free radical species may be sometimes the product of normal circular metabolism and at low concentration regulate physiological function of cells [35, 80]. The first cell type reported to produce free radicals was

sperm [54]. Sperm membranes are rich in polyunsaturated fatty acids, which make them very susceptible to oxygen-induced damage mediated by lipid peroxydation [74]. Low level of production of ROS by sperm supports some main functions, such as capacitation, acrosome reaction, zona pellucida binding and oocyte fusion [22]. The high production of ROS, is one of the reason of sperm aberration leading to infertility.

The disturbances in the balance between the concentration of ROS and the removing of them by antioxidant scavenging system cause "oxidative stress", which seems to be an important factor, leading to diminish sperm function through peroxidative damage to the cell membrane. Moreover, oxidative stress induces DNA strands breaks and affects proteins [1, 58, 76].

RSV increases the action of superoxide dismutase, which reduces superoxide to hydroxen peroxide, but the level of hydroxen peroxide is not elevated due to other cellular activity [65] .

Conflicting data are present in relation to activity of RSV in free radical scavenging, dealing with its ability to scavenge hydroxyl radical or stable free radicals [33, 52], but also to increase the level of oxidative DNA strand breaks through the induction of copper-peroxide complexes [13, 88]. RSV is believed to complete with coenzyme Q and to decrease the oxidative chain III, the site of ROS generation. Moreover, RSV scavenges superoxide radicals formed in mitochondria and inhibits lipids peroxidation induced by Fenton reaction products [91] and reduces of nitric oxide synthase to prevent cytotoxic effect [44, 55, 81].

Depending on the individual based human DNA status, used assays, doses and observed endpoints, RSV was able switch from antioxidant to pro-oxidant [23, 79] and may act as an efficient and presumably radical scavenger [43]. Usually, at lower doses RSV act as an antioxidant, whereas at higher dose it may act as pro--oxidant RSV was found to be an effective scavenger hydroxyl, superoxide and metal-induced radicals as well as having antioxidant abilities in cells producing ROS. It exhibits a protective effects against lipid peroxidation in cell membranes and DNA damage caused by ROS [4, 9, 67]. RSV regulates the redox homeostasis in mammalian system by maintaining the amounts of several antioxidant enzymes, including glutatione peroxidase, glutathione S-transverase and glutatione reductase [90].

RESVERATROL AND INFERTILITY

Resveratrol seems to be promising new compound for the treatment of male infertility. RSV however structurally similar to estrogens showed no estrogenic properties and did not affect testis contrary to other estrogen-like compounds [46, 51]. It modulates the estrogenic response system and may be involved in male reproduction [46]. RSV decrease germ cells apoptosis in mice and rats and play protective role on the male reproductive tract [63, 82] as well as enhances blood testosterone levels, testicular sperm count and epididymes sperm motility in rabbits [72]. ICR mice given 50 mg/kg of resveratrol daily for 28 days showed increase in the relative weights of the testes and epididymides and normal features of testis, as well as enhanced epididymal sperm motility and testicular sperm count [72]. Contrary, other studies show that RSV may cause reduction in testicular weights impairs seminiferous tubules morphology and spermatogenesis [37, 40, 70]. *Garcez* et al. [39] reported that RSV protects human sperm against oxidative damage, however not against the loss of motility induced by cryptopreservation. In turn, *Branco* et al. [11] observed that RSV prevent DNA damage caused by cryptopreservation of human semen both fertile and infertile men. Such finding is very important for successful fertilization and normal embryo development. Studies on rats showed that RSV enhance sperm production by stimulating the hypothalamic-pituitary-gonadal axis without inducting adverse effects [46]. RSV affects the stimulation of cell proliferation of prostate tumor cells in vitro and affect the production of testosterone in a biphasic manner. These observations were associated with the capacity of resveratrol to enhance sperm production in healthy animals. Sperm count were significantly (about 1.7 times) higher in the resveratrol treated male rats compared to control group, however the sperm quality did not differ [46]. The increase in the sperm production observed following RSV administration may be also caused by an overall increase in the size of spermatogenic tissue (decrease in the mean diameter of the seminiferous tubules with increase in the testicular tubules density). Sperm motility increased progressively at 30μM, 15 μM and 6 μM of RSV. Lower doses of RSV act against lipid peroxidation, preserving sperm chromatin and plasma membranes [46]. The scavenger properties of RSV was demonstrated in vitro in human sperm and on rat germ cells [18]. RSV at 1 mg/kg and 10 mg/kg improved the sperm motility and enhanced antioxidant defenses in testis of hyperthyroid rats [60]. Moreover, RSV harden the recovery of testis weight and cause restart of spermatogenesis process in rats after testicular injury caused by 2,4-hexanedione [41]. It is a potent inhibitor of the oxidation of polyunsaturated fatty acids found in lipoprotein [59]. Therefore RSV could be active by decreasing the level of ROS and proinflammatory factors in seminiferous tubules, thus increasing sperm and androgen production [46].

THE EFFECT OF RESVERATROL IN A COMBINATION WITH OTHER AGENTS

There is known that RSV acting in combination with other agents inhibit the toxic effects of them.

Supplementation with resveratrol reduced oxidative damage induced by acrylamide to DNA of somatic and germ cells of rats [2]. Pretreatment with RSV significantly inhibited ethanol induced oxidative DNA-damage in human peripheral lymphocytes [89]. Similarly, pretreatment of whole blood sample with RSV before incubation with iodine-131 reduced frequency of micronuclei compared to ^{131}I alone [42]. RSV could protect cells from UVB-radiation much better when used in lower dose. In case of UV radiated HEK 293 cells it was shown that 100 M RSV suppressed cell proliferation and cell showed loss of membrane integrity, whereas the dose of 10 M RSV increase cell proliferation and the number of cells without any evidence of toxicity [14]. RSV showed a protective effect against UVA irradiation in cultured RPE cells in the pretreatment model, but not in posttreatment model [71].

Less is known about the ability of resveratrol to modify the effect of radiation exposure in normal and cancer cells. However, there are evidences that, RSV is able to modulate the behavior of cells in response to radiation-induced damage. RSV is unable to produce DNA damage detectable by comet assay and modulates the radiation induced effects in cell cycle as well as in apoptosis [3, 34]. Pretreatment with RSV protects mouse embryonic stem cells from DNA damage induced by ionizing radiation [24]. RSV mitigates the apoptotic clearance of irradiated cells and prevents the G2 phase cell cycle arrest induced by X-rays [34].

The pretreatment with the dose of 100 mg/kg RSV, 2 days prior single irradiation to 3 Gy significantly reduced the mean total chromosome aberration frequency in samples taken at 1 and 30 days after whole-body γ-radiation [5, 15]. *Baatout* et al. [5] noted that RSV enhanced radiation-induced apoptosis of cancer cell lines. As recent studies reported RSV increased radiosensitivity of cancer cells by inhibitory of cell proliferation and enhanced apoptosis [31, 32, 78].

CONCLUSIONS

Considering that controlled exposure to ionizing radiation is one of the most effective treatments of cancer patients, agents that improve the efficiency of radiation killing of cancer cells and prevent the damage to normal cells and tissues are needed. RSV, which is non-toxic and already commercially available in pills, might be efficiently used in the radioprotection with preventive and/or therapeutic effects. The antioxidant properties and ability to induce apoptosis and cell cycle arrest as well as lack of toxicity make resveratrol an attractive candidate for radioprotection of normal cells and cancer prevention.

Minimization of radiation induced damage to somatic and germ cells by resveratrol administration might be useful also in case of radiological accidents and might contribute to public health prevention and also to reduction of public trouble.

Both during radiological accidents and treatments of cancer patients, especially important seems to protection of germ cell. In case of creation of germ cell mutations following DNA damage, such mutations can be passed to the offspring of irradiated person and onward through future generations. For this reason using of RSV is also promising.

Results of previous studies showed that RSV has ability to decrease harmful effects of radiation, so it seems to be promising for radioprotection.

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