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Effect of DNA repair deficiencies on the cytotoxicity of resveratrol

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Effect of DNA repair deficiencies on the cytotoxicity of resveratrol

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Abstract

Numerous preclinical studies have shown that the naturally-occurring polyphenol resveratrol may produce health-beneficial effects in a variety of disorders, including cancer, diabetes, Alzheimer, and cardiovascular diseases. Resveratrol has entered clinical trials for the prevention and treatment of several of these disorders. This polyphenol is also available in the market as a dietary supplement. Experimental data have shown, however, that resveratrol induces DNA damage in a variety of cells. Here we review such evidence and evaluate the cytotoxicity of resveratrol (MTT assay) in cells deficient in several major DNA repair pathways (i.e., homologous recombination, non-homologous end joining, base excision repair, nucleotide excision repair, mismatch repair, and Fanconi anemia repair). Cells deficient in base excision repair (EM9), nucleotide excision repair (UV4 and UV5) and Fanconi Anemia (KO40) were slightly hypersensitive to resveratrol-induced cytotoxicity with respect to their parental cells (AA8). Our results suggest that these pathways may participate in the repair of the DNA damage induced by resveratrol and that deficiencies in these pathways may confer hypersensitivity to the genotoxic activity of this dietary constituent.

Introduction

Numerous studies have been conducted to assess the possible health benefits of resveratrol. Preclinical studies suggest that this dietary occurring stilbene may confer protection against metabolic, cardiovascular and other age-related complications, including neurodegeneration and cancer (1-19). Many clinical trials have been conducted, or are ongoing or recruiting participants, to evaluate the health benefits of resveratrol in humans (<https://clinicaltrials.gov/>). These trials have not shown relevant health benefits so far. Because resveratrol is a natural constituent of grapes and because grape consumption is not associated with any type of toxicity, it is believed that the potential health benefits of this compound will be achieved without toxicity. This may explain why resveratrol is readily available in the market and is

widely consumed as a dietary constituent.

Large cancer chemoprevention clinical trials have demonstrated, however, that some dietary constituents (i.e. beta-carotene, vitamin E) significantly increased cancer risk (lung and prostate cancer, respectively) when used in the form of dietary supplements (20;21). The toxicity of some dietary constituents may be mediated by their prooxidant and DNA damaging activities (22-25). Inhibition of DNA topoisomerase activity may also lead to genotoxic damage, and several dietary constituents induce topoisomerase-mediated DNA damage (23-43). Although resveratrol is generally regarded as a safe antioxidant, evidence suggests that resveratrol may also induce genotoxicity, which may be mediated by its pro-oxidant and topoisomerase poisoning activities (27;30;37;38;44;45). Table 1 shows several reports in which the DNA-damaging activity of resveratrol has been evaluated in cells.

The DNA damage effect of resveratrol could be enhanced in cells with specific DNA repair deficiencies. For example, BRCA2 is a DNA repair gene involved in the repair of double-strand DNA breaks. People with mutations in BRCA2 have an increased cancer risk because they are unable to properly repair this type of DNA damage. These individuals would be hypersensitive to the DNA damaging activity of dietary agents that induce double-strand DNA breaks. People without inherited mutations in DNA repair genes may develop sporadic mutations in these genes during a possible carcinogenesis process, and may therefore become hypersensitive to dietary agents inducing specific types of DNA damage. It is important, therefore, to evaluate the cytotoxicity of common dietary constituents in cells lacking an intact DNA damage repair capacity.

Several major DNA repair pathways are known to participate in the repair of most types of DNA damage. These pathways include homologous recombination (HR) repair, non-homologous end joining (NHEJ) repair, base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), Fanconi anaemia (FA) repair. The choice of each of these pathways mainly depends on the type of DNA damage that occurs, although other factors (such as the phase of the cell cycle) are known to play a role. Most of these pathways incorporate a similar set of

coordinated processes that include the detection of DNA damage, the accumulation of DNA repair factors at the site of damage and, finally, the physical repair of the lesion (46;47). HR is involved in the repair of DNA double-strand breaks, DNA inter-strand cross links and damage encountered during DNA replication. NHEJ is also involved in the repair of DSBs. In contrast to HR, NHEJ ligates the two ends of a DSB without the requirement of a homologous template. NHEJ is error prone, because this process can cause the deletion or mutation of nucleotides around the DSB site. NHEJ occurs throughout the cell cycle, but mainly in the G0/G1 phases. The BER pathway repairs damaged bases (a single base or a short strand containing a damaged base), abasic sites and single strand breaks. Whereas BER repairs small base damages, NER repairs some of the bulkier DNA lesions that distort the DNA helical structure, such as those induced by UV radiation and by some chemical carcinogens. NER is divided into two sub-pathways: the global genome NER (GG-NER) and the transcription coupled NER (TC-NER). GG-NER identifies and removes damages all over the genome whereas TC-NER repairs actively transcribed genes. Mismatch repair (MMR) plays an important role in maintaining genomic stability by repairing small insertions, deletions and base-base mismatches caused by spontaneous and induced base deamination, oxidation, methylation and replication errors. Fanconi Anemia (FA) is a disorder characterized by chromosomal instability, cancer susceptibility, and a profound sensitivity to agents that produce DNA interstrand cross-links. The FA pathway is thought to mediate the repair of DNA interstrand cross-links at replication forks, possibly by facilitating ATR- and ATM-dependent checkpoint signaling and activating HR repair (46;47). In this work, we have used cell lines with defects HR, NHEJ, BER, NER, MMR and FA to assess the effect of specific DNA repair deficiencies on the cytotoxicity of resveratrol.

Methods

Chemicals and Cell lines

Resveratrol was purchased from Sigma. The effect of resveratrol was studied in cells deficient in the following DNA repair pathways: Homologous recombination (HR), Non-homologous recombination (NHEJ), Base excision repair (BER), Nucleotide excision repair (NER), Mismatch repair (MMR) and Fanconi anemia repair (FA) (47). Table 2 shows additional information on these DNA repair deficient cell lines and on their parental non-deficient

counterparts. The cell lines were maintained in DMEM supplemented with 2 mM glutamine, 50 µg/mL penicillin, 50 µg/mL streptomycin and 10% fetal bovine serum, and were cultured at 37°C in a humidified atmosphere containing 5% CO₂ (48;49).

MTT cell proliferation assay

The MTT assay is a colorimetric technique that allow the quantitative determination of cell viability (50). It is based on the capability of viable cells to transform the MTT salt (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) into formazan dye. Exponentially growing cells were seeded into 96-well plates and resveratrol was added 24 h later. After a drug exposure period of 48h, cells were incubated with MTT for 5h. Then, 80 µl 20% SDS in 0.02 M HCl were added, and the plates were incubated for 10 h at 37°C before measuring optical densities at 540 nm on a multiwell plate spectrophotometer reader. Cell viability was expressed as percentage in relation to controls. For statistical analysis we used the t-test (paired, two-tailed). All data were averaged from at least two independent experiments and were expressed as the means ± standard error of the means (SEM).

Results and Discussion

Evidence has accumulated that some dietary constituents may induce toxicity when used at higher concentrations than those achieved in a normal diet. For example, since the antioxidant agent beta-carotene is found in vegetables and fruits and because eating vegetables and fruits is associated with a reduced risk of cancer, it seemed reasonable to think that taking high doses of beta-carotene supplements might reduce cancer risk. Preclinical studies supported the possible cancer preventive activity of this dietary constituent. In three major clinical trials, people were given high doses of beta-carotene (20-30 mg, which are approximately 10-times higher than those found in a diet rich in fruits and vegetables) in an attempt to prevent lung cancer and other cancers. These trials were stopped ahead of schedule because two of them found beta-carotene supplements to be associated with a higher risk of lung cancer (20). It has also been found in another major chemoprevention trial that dietary supplementation with vitamin E significantly increased the risk of prostate cancer among healthy men(21). The pro-oxidant activity of beta-carotene and vitamin E may account for their toxicity, because pro-oxidant compounds can increase the cellular levels of reactive oxygen species (ROS), which are known to induce carcinogenic effects. The inhibition of DNA

topoisomerase is also known to lead to carcinogenic effects, and some dietary constituents are able to induce topoisomerase-mediated DNA damage (25;51;52).

Because resveratrol can both induce pro-oxidant activity and inhibit DNA topoisomerase function (27;30;37;38;44;45), and because these activities can lead to DNA damage, it is not surprising that this dietary agent can induce DNA damage in a variety of cells (Table 1). Some reports show that resveratrol induces DNA damage in the low micromolar range (Table 1). Human studies have shown that supplementation of resveratrol leads to plasma concentrations in the nanomolar-micromolar range (53-55). This suggests that some tissues may be exposed to carcinogenic concentrations of resveratrol after dietary supplementation. This effect may be more pronounced in people with heritable defects in genes involved in DNA damage repair. During a possible carcinogenic event, premalignant cells may acquire mutations in DNA repair genes. This would make them hypersensitive to the carcinogenic activity of compounds that induce types of DNA damage that require these DNA repair genes for repair. Therefore, we considered relevant to evaluate the effect of specific DNA repair deficiencies on the cytotoxicity of resveratrol.

Our results show that cells lacking particular DNA repair genes are slightly but significantly hypersensitive to the cytotoxicity of resveratrol (Figure 1). In some cases, differences between the deficient cell line and the parental cell line were observed in the low micromolar range. These differences were mild and require further investigation. However, our preliminary findings support the view that the supplementation of resveratrol at high concentrations may induce carcinogenic effects under specific conditions.

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Illustrations

Illustration 1

Table 1

Induction of cellular DNA damage by resveratrol

U87-MG glioblastoma cells: 0.5h, 120µM, ICE Bioassay (+: DNA damage was observed)	Leone,S., Basso,E., Polticelli,F. and Cozzi,R. (2012) Resveratrol acts as a topoisomerase II poison in human glioma cells. <i>Int. J. Cancer</i> , 131, E173-E178.
Human lymphocytes: 2h, 200µM, Comet assay (+)	Azmi,A.S., Bhat,S.H., Hanif,S. and Hadi,S.M. (2006) Plant polyphenols mobilize endogenous copper in human peripheral lymphocytes leading to oxidative DNA breakage: a putative mechanism for anticancer properties. <i>FEBS Lett.</i> , 580, 533-538
Murine leukemia (L1210) cells: 24h, 1.25, 2.5 and 5µM, Comet assay (+) Human myelogenous leukemia (K562) cells: 24h, 1.25, 2.5 and 5µM, Comet assay (+) Human promyelocytic leukemia (HL-60) cells: 24h, 1.25, 2.5 and 5µM, Comet assay (+)	Ovesna,Z., Kozics,K., Bader,Y., Saiko,P., Handler,N., Erker,T. and Szekeres,T. (2006) Antioxidant activity of resveratrol, piceatannol and 3,3',4,4',5,5'-hexahydroxy-trans-stilbene in three leukemia cell lines. <i>Oncol. Rep.</i> , 16, 617-624.
Human epidermoid carcinoma (A431) cells: 1h, 100µM, ICE Bioassay (+); 1h, 1, 10 and 50µM, Comet assay (-)	Baechler,S.A., Schroeter,A., Dicker,M., Steinberg,P. and Marko,D. (2014) Topoisomerase II-targeting properties of a grapevine-shoot extract and resveratrol oligomers. <i>J. Agric. Food Chem.</i> , 62, 780-788.
Human glioblastoma (U87-MG) cells: 48h, 20-80 µM, γ-H2AX (Immunofluorescence and Flow cytometric) (+)	Leone,S., Cornetta,T., Basso,E. and Cozzi,R. (2010) Resveratrol induces DNA double-strandbreaks through human topoisomerase II interaction. <i>Cancer Lett.</i> , 295, 167-172.
Chinese hamster lung (CHL) cells: 24 and 48h, 11-88 µM, Micronucleus assay (+)	Matsuoka,A., Furuta,A., Ozaki,M., Fukuhara,K. and Miyata,N. (2001) Resveratrol, a naturally occurring polyphenol, induces sister chromatid exchanges in a Chinese hamster lung (CHL) cell line. <i>Mutat. Res.</i> , 494, 107-113.
Chinese hamster V79 cells: 6h+11h recovery, 100 µM, Micronucleus assay (+) Mouse lymphoma L5178Y cells: 4h+20h recovery, 1-60µM, Micronucleus assay (+)	Schmitt,E., Lehmann,L., Metzler,M. and Stopper,H. (2002) Hormonal and genotoxic activity of resveratrol. <i>Toxicol. Lett.</i> , 136, 133-142.
Chinese Hamster Ovary (CHO) K1 cells: 6h+22h recovery, 40, 80 and 120µM, Micronucleus assay (+)	Basso,E., Fiore,M., Leone,S., Degrassi,F. and Cozzi,R. (2013) Effects of resveratrol on topoisomerase II-α activity: induction of micronuclei and inhibition of chromosome segregation in CHO-K1 cells. <i>Mutagenesis</i> , 28, 243-248.

Illustration 2

Figure 1

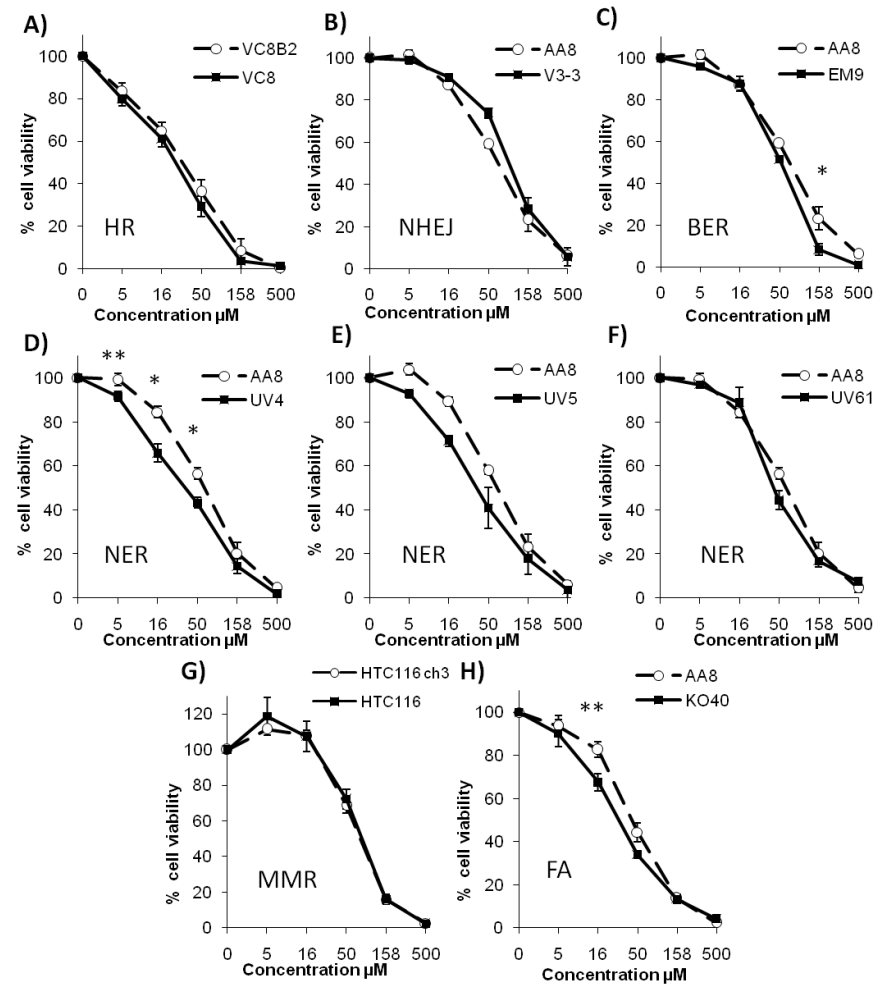


Figure 1: Cytotoxicity of Resveratrol in cells deficient in several DNA repair pathways. Cells were exposed to resveratrol for 48h and cell viability was estimated with the MTT assay. A) Homologous recombination deficient cells VC8 *versus* parental cells VC8-B2; B) Non homologous end joining deficient cells V3-3 *versus* parental cells AA8; C) Base excision repair deficient cells EM9 *versus* parental cells AA8; D-F) Nucleotide excision repair cells UV4, UV5 and UV61 *versus* parental cells AA8; G) Mismatch repair deficient cells HTC116 *versus* parental cells HTC116 ch3; H) Fanconi deficient cells KO40 *versus* parental cells AA8. Data show the mean and the SD from at least 2-3 independent experiments. Differences were statistically significant differences (Student's t-test): *P<0.05, **P<0.01

Illustration 3

Table 2

DNA REPAIR PATHWAYS	CELL LINES
HR	VC8: V79 Chinese hamster lung cells mutated in BRCA2. HR deficient. VC8B2: VC8 cells complemented with human BRCA2 (functioning HR, parental).
NHEJ	AA8: Parental Chinese hamster ovary cells. V3-3: AA8 cells mutated in XRCC7 (human homologous DNA-PK). NHEJ deficient.
BER	AA8: Parental Chinese hamster ovary cells. EM9: AA8 cells mutated in XRCC1 (DNA ligase III). BER deficient.
NER	AA8: Parental Chinese hamster ovary cells. UV4: AA8 cells mutated in ERCC1 (XPF). NER deficient. UV5: AA8 cells mutated in ERCC2 (XPD). NER deficient. UV61: AA8 cells mutated in ERCC6 (CSB). NER (transcription coupled) deficient.
MMR	HTC116+ch3: HTC116 complemented with chromosome 3 (with MLH1 gene; functioning MMR, parental) HTC116: Human colon cancer cells mutated in MLH1. MMR deficient.
FA	AA8: Parental Chinese hamster ovary cells. KO40: AA8 cells mutated in FANCG (FANCG). FA deficient.

Table 2: Cell lines used in the present study. HR: homologous recombination; NHEJ: non homologous end joining; BER: base excision repair; NER: nucleotide excision repair; MMR: mismatch repair; FA: Fanconi anemia repair.