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Leukocyte 8-oxo-7,8-dihydro-2′-deoxyguanosine and comet assay in epirubicin-treated patients

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Abstract

Epirubicin fights cancer through topoisomerase II inhibition, hence producing DNA strand breaks that finally lead to cell apoptosis. But anthracyclines produce free radicals that may explain their adverse effects. Dexrazoxane—an iron chelator—was proven to decrease free radical production and anthracycline cardiotoxicity.

In this article, we report the concentrations of cellular 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dGuo) relative to 2′-deoxyguanosine (dGuo), and comet assay results from a study including 20 cancer patients treated with epirubicin. Plasma concentrations of vitamins A, E, C and carotenoids are also reported. All data were obtained before and immediately after epirubicin infusion. The ratios of 8-Oxo-dGuo to dGuo were measured in leukocyte DNA by HPLC-coulometry after NaI extraction of nucleic acids. Vitamins A and E and carotenoids were measured by HPLC-spectrophotometry. Vitamin C was measured by HPLC-spectrofluorimetry.

Median 8-oxo-dGuo/dGuo ratios increased significantly from 0.34 to 0.48 lesions per 100,000 bases ($p = 0.002$) while per cent of tail DNA increased from 3.47 to 3.94 after chemotherapy ($p = 0.001$). 8-Oxo-dGuo/dGuo and per cent of tail DNA medians remained in the normal range. Only vitamin C decreased significantly from 55.4 to 50.3 μM ($p = 0.013$). Decreases in vitamins A, E, lutein and zeaxanthin were not significant, but concentrations were below the lower limit of the normal range both before and after chemotherapy. Only the correlation between comet assay results and vitamin C concentrations was significant ($\rho = -0.517$, $p = 0.023$).

This study shows that cellular DNA is damaged by epirubicin-generated free radicals which produce the mutagenic modified base 8-oxo-dGuo and are responsible for strand breaks. However, strand breaks are created not only by free radicals but also by topoisomerase II inhibition. In a previous study we did not find any significant change in urinary 8-oxo-dGuo excretion after adriamycin treatment. However, 8-oxo-dGuo may have increased at the end of urine collection as DNA repair and subsequent kidney elimination are relatively slow processes. In another study, authors used GC-MS to detect 8-oxo-dGuo in DNA and did not find any change after prolonged adriamycin infusion. Reasons for these apparent discrepancies are discussed.

Keywords: *Anthracycline, DNA damage, vitamin A, vitamin C, vitamin E, carotenoids*

Epirubicin belongs to anthracyclines, a family of potent anticancerous drugs [1] that act by inhibiting topoisomerases which are indispensable for DNA replication and cell division [2,3]. DNA topoisomerases are nuclear enzymes, which induce transient DNA breaks thus allowing DNA strands or double helices to pass through each other. Type I enzymes induce single stranded cuts in DNA, and type II enzymes cut and cross through double stranded DNA. DNA toposisomerases regulate DNA coiling and supercoiling necessary for DNA to be stored in a small volume in non-dividing cells, and allow the release of supercoiled DNA that is required for its transcription. Anthracyclines must

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intercalate into the DNA coils to be active [4] and to stabilise an intermediate reaction in which DNA strands are cut and covalently linked to tyrosine residues of the enzyme. Since anthracyclines act on cell division, their primary targets are perpetually dividing tissues and especially cancerous tissues.

Besides their anticancerous action, anthracyclines are also potent free radical generators. Indeed, the reduction of the central quinone gives rise to a semiquinone free radical. Flavoproteins favour the formation of semiquinone radicals by taking up electrons from NADH and NADPH and transferring them to anthracyclines. Reduction of oxygen to superoxide regenerates the parental anthracycline molecule [5]. In the presence of superoxide dismutase (SOD), superoxide can form hydrogen peroxide. The semiquinones can bind iron and thus produce the highly toxic $^{\cdot}OH$ from superoxide and hydrogen peroxide through the Haber–Weiss cycle. $^{\cdot}OH$ radicals cause DNA damage [6], lesions to membranes and to various cell structures [7]. Free radicals generated by anthracyclines are thought to play a major role in their adverse effects, particularly in cardiomyopathy [8–11] and chemotherapy-induced leukaemia [12,13] that occur in anthracycline-treated patients. Dexrazoxane—an iron chelator—decreases iron-mediated free radical formation thus protecting the heart from the effects of anthracyclines without decreasing their effectiveness on cancer [14–16]. Epirubicin is a semisynthetic derivative of doxorubicin that produces fewer free radicals while preserving its anticancer action [17]. These facts, and the experiments of Liu et al. [18] who found that free radicals were not involved in the action of Idarubicin on leukaemia cells, suggest that free radicals play only a small role in the anticancer action of anthracyclines.

Electron micrographs show that cardiomyocyte damage due to epirubicin consists of loss of myofibrils together with separation of the intercalated disk and dilation of the sarcotubular system [19]. In a cultured cardiomyocyte model, the antioxidants α-phenyl-tert-butyl-nitrone, trolox and 5-aminosalicylic acid provided protection against the oxidative stress induced by adriamycin [20]. Kumar et al. [21] reported that adriamycin increased the number of apoptotic cardiomyocytes, caused nucleosomal fragmentation and DNA ladder formation. These authors found that these alterations were reduced by trolox and suggest that these were mediated by free radicals. These findings are consistent with a deleterious role of anthracyclines through DNA damage, which is subsequent to oxygen radical attacks on the nucleic acids.

8-Oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dGuo), the C-8 product of 2′-deoxyguanosine (dGuo) hydroxylation, is one of the most frequent oxidative lesions to DNA [22]. Hydroxyl radicals generate multiple products from all four bases, whereas singlet oxygen preferentially alters dGuo, particularly through 8-hydroxylation [23,24]. 8-Oxo-dGuo is a mutagenic DNA alteration as it forms G-T and A-C substitutions [25], and is able to activate oncogenes [26]. The comet assay detects strand breaks produced by hydroxyl radicals, and also the few strand breaks produced by topoisomerase inhibition.

In this paper, we compare and discuss the measurement of two DNA lesions—8-oxo-dGuo expressed as a concentration relative to that of dGuo, and DNA strand breaks detected by the comet assay—in 20 cancer patients who were treated with epirubicin. Urinary oxidative DNA lesions have previously been reported in adriamycin-treated patients [27], but 8-oxo-dGuo and comet assay data have never been compared in the cellular DNA of adriamycin-treated patients. This study is aimed to assess cellular DNA damage that could be caused by epirubicin treatment and to investigate the influence of antioxidant vitamins on the extent of DNA damage.

Patients, materials and methods

Patients

Twenty female patients presented with breast cancer were included in the study, and 5 ml of blood were drawn before and after the 2-h venous epirubicin infusion. All patients received 100 mg epirubicin/m^2 of body area, except two who received only 75 mg/m^2. To avoid cumulative effects, samples were collected during the first course of epirubicin. Epirubicin was combined with cyclophosphamide in all patients (715–1000 mg) and 5-fluorouracil (715–1000 mg). Patients were 41–73 years of age (median 54.5 years); their mean BMI was 25.5 (SD = 4.87).

Controls were 15 healthy women from the laboratory staff, 35–65 years of age.

Procedures used in this study strictly complied with the Helsinki conference guidelines for research on human beings. The patient's routine treatment was not modified by the present study.

Materials

Apparatus. Vitamins were measured with a Biotek-Kontron HPLC system (Biotek-Kontron, Montigny le Bretonneau, France) which consisted of a 525 gradient pump, 482-column oven, a 565 auto-sampler, a 540-diode array detector, an SFM 25 fluorescence detector and a Geminyx data station.

8-Oxo-dGuo and dGuo were measured with a Biotek-Kontron HPLC system which consisted of a 422 pump, a pulse dampener, a 482 column oven, a 460 auto-sampler, an ESA Coulochem II detector (ESA, Bedford, MA, USA) equipped with a 5011 cell, a Jasco UV1565 detector (Jasco France, Nantes), and a 450 Data System.

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Comet assays were performed with a Zeiss fluorescence microscope under green light. The microscope was connected to a charge coupled device (CCD) camera and a computer based analysis system (Comet Analysis Software, version 4.0, Kinetic Imaging Ltd., Liverpool, UK) to determine the extent of DNA damage after electrophoretic migration of DNA fragments in agar.

Reagents. Fat-soluble vitamins and carotenoids were separated with a 3 μ Adsorbosphere HS C18 150 × 4.6 ID column (Alltech Associates, Inc. Deerfield IL, USA), and vitamin C was analysed with a 5 μ Nucleosil 100 AB 150 × 4.6 mm ID column (Macherey Nagel Sarl, Hoerdt, France). 8-Oxo-dGuo and dGuo were separated with an Inertsil ODS 3 250 × 4.6 mm ID column (GL Science, Tokyo, Japan).

Ultrafiltration was run using Amicon YMT 30 membranes and MPS-1 supporting devices (Grace-Amicon, Beverly, MA, USA) to remove proteins and high MW material's before HPLC analysis.

Methanol, acetonitrile, dichloromethane, hexane, tetrahydrofuranne and ethanol were purchased from Riedel-de Haën (Sigma-Aldrich Corp, St Louis, MI, USA). Retinol, α-tocopherol, β-carotene, 8-oxo-dGuo, dGuo, RNAse IIIa, RNAse T1 and Nuclease P1 were purchased from Sigma (Sigma Aldrich Chemicals, St Louis, USA). Alkaline phosphatase was purchased from Roche (Roche Applied Science, Meylan, France).

Low-melting temperature agarose was purchased from FMC Bioproducts (Rockland, ME, USA), sodium sarcosinate, ethylendiaminetetracetic acid disodium salt (Na_2 EDTA), Tris base, Triton 100 × and ethidium bromide were purchased from Sigma Chemical Company (St. Louis, MI), phosphate buffered saline (PBS) without calcium and magnesium, and RPMI 1640 medium were purchased from Gibco (Grand Island, NY)

Methods

Blood sampling. Two millilitres of blood were collected from each patient in heparinised tubes before and after the 2-h drug infusion. Blood was rapidly centrifuged at 2000*g* for 10 min, and plasma was aspirated carefully, and then frozen at −80°C for fat-soluble vitamin measurements. For vitamin C measurements, a plasma aliquot was immediately mixed with an EGTA-Glutathione solution. All aliquots were frozen and kept at −80°C until analysis.

Blood cells were washed in three volumes of NaCl solution (0.9%), then re-centrifuged at 4°C for 10 min and 1000*g*. Red cells were then lysed with four volumes of a Tris–EDTA buffer (20 mM, pH 8) and kept at 0°C for 10 min. Then a first leukocyte pellet was obtained after a 10-min centrifugation. The pellet was washed again with 25 ml of the Tris–EDTA buffer and re-centrifuged. Finally the clean leukocyte pellet was dispersed in 1 ml of 0.9% NaCl and frozen until 8-oxo-dGuo measurement.

Five hundred microlitres of blood were taken from all volunteers by venipuncture, immediately stabilised with 500 μl of a mixture of dimethylsulfoxide (DMSO)/cell culture medium (RPMI 1640) and frozen to −80°C until analysis by comet assay according to Hininger [28].

Vitamin measurements. Fat-soluble vitamins and carotenoids were measured by reversed-phase HPLC and diode-array detection using the Steghens method [29], and HPLC with fluorescence detection was used to measure total plasma vitamin C [30].

Measurement of 8-oxo-dGuo and dGuo in leukocyte DNA. DNA 8-oxo-dGuo in leukocytes was measured by HPLC and coulometric detection using the general methods described by the ESCODD group [31]. Leukocyte membranes were dissolved at pH 7.5 with 5 mM $MgCl_2$ and 1% Triton X100. RNAs were then removed with 50 μg of RNAse III per ml and 10 IU RNAse T1 per ml. Cell structures were further destroyed with 1 mg protease K per ml.

Cell DNA was extracted at pH 8, in the presence of desferrioxamine, NaI 4 mM, and isopropanol, then washed once with 2 ml isopropanol and then with 2 ml 70% ethanol. DNA was subsequently dissolved in 175 μl of a 100-μM desferrioxamine solution.

DNA was hydrolysed for 1 h with 10 U of nuclease P1 at 37°C. Nucleotides were dephosphorylated using 8 U of alkaline phosphatase for 1 h at 37°C. Proteins were removed by ultrafiltration with the MPS-1 kits. The nucleoside solution was then analysed by HPLC. 8-Oxo-dGuo was measured in the column eluate by coulometry with a 190-mV potential applied on the first electrode and a 310-mV potential on the second electrode, but these voltages could differ slightly from one electrode to another. Voltamograms were made once a month to obtain maximum sensitivity and specificity. dGuo was measured by spectrophotometry at 280 nm.

The mobile phase was a 50-mM pH 5.5 phosphate buffer, which contained 12% methanol. HPLC separations were run at 26°C, under these conditions, 8-oxo-dGuo eluted at 12 min and dGuo at 9 min.

Comet assay. After a quick thawing in a water bath at 37°C, blood samples were centrifuged at room temperature at 300*g* for 5 min. The cell pellet was washed twice and then dispersed in a Ca^{++} and Mg^{++} free PBS solution to obtain about 20,000 cells

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Table I. Markers of DNA damage, serum vitamins and carotenoids in cancer patients before and after chemotherapy (Wilcoxon rank test).

	Before chemotherapy			After chemotherapy			
	5th percentile	Median	95th percentile	5th percentile	Median	95th percentile	p
8-Oxo-dGuo/10^5 dGuo	0.037	**0.340**	1.16	0.128	**0.480**	1.30	**0.002**
Comet (%tail DNA)	2.90	**3.47**	6.34	3.18	**3.94**	10.7	**0.001**
Vitamin C (μM)	6.90	**55.4**	77.1	5.10	**50.3**	76.7	**0.013**
Vitamin A (μM)	0.785	**1.39**	2.14	0.713	**1.29**	2.17	0.478
Vitamin E (μM)	16.9	**25.5**	44.5	17.9	**23.5**	41.3	0.211
Lutein (μM)	0.0631	**0.225**	0.472	0.0621	**0.204**	0.474	0.332
Zeaxanthin (μM)	0.0420	**0.0430**	0.0895	0.00710	**0.0420**	0.129	0.777
β-Cryptoxanthin (μM)	0.0644	**0.182**	0.537	0.121	**0.182**	0.519	0.112
Lycopene (μM)	0.114	**0.385**	1.18	0.113	**0.366**	1.17	0.147
α-Carotene (μM)	0.0342	**0.139**	0.313	0.0361	**0.127**	0.285	0.387
β-Carotene (μM)	0.113	**0.517**	3.14	0.125	**0.517**	3.08	0.227

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in 60 μl. Comet assays were performed according to Singh et al. [32], with minor modifications. Thus, 10 μl of fresh or thawed blood were mixed with 110 μl of 0.6% low melting point agarose in RPMI 1640 at 37°C. Subsequently, 110 μl of this mixture was layered onto a coded slide (pre-coated with a thin layer of 1% agarose and dried), and immediately covered with a cover glass. Slides were left for 10 min on ice to allow the agarose to solidify. Gently after removing the coverglass, slides were immediately immersed in an ice-cold freshly prepared lysis solution (2.5 M NaCl, 10 mM Na_2-EDTA, 10 mM TRIS hydroxymethyl–aminomethane, 1% sodium sarcosinate, 1% Triton 100 × and 10% DMSO, pH 10) to lyse the cells and to allow DNA unfolding. After 1 h in the dark at 4°C, the slides were immersed in a fresh alkaline electrophoretic solution (300 mM NaOH, 200 mM Na_2-EDTA, pH 13) for unwinding (25 min) and then electrophoresed (25 V / 300 mA, 25 min). The electrophoretic tank was covered with black paper to avoid any additional light-induced DNA damage. After electrophoresis completion, gels were neutralised in 0.4 M Tris, pH 7.5. The slides were then stained with ethidium bromide, covered prior to analysis with a fluorescence microscope under green light. Results were expressed as percentages of DNA in the tail (% tail DNA).

Statistical analysis. Results were analysed with the SPSS for Windows package (SPSS Chicago, IL, USA) using Wilcoxon tests and the Spearman correlation coefficient. The non-normal distributions of most of the measured parameters did not allow us to use parametric tests. Differences were considered significant for $\alpha \leq 0.05$.

Results

Results concerning markers of DNA damage, vitamins and carotenoids are summarised in Table I, and Table II presents the range of these parameters as observed in healthy controls. In addition, Figure 1 shows the individual variations of 8-oxo-dG and per cent tail DNA in patients before and after chemotherapy.

8-Oxo-dGuo/dGuo ratios and per cent tail DNA increased significantly, but median differences were larger for comet assays. 8-Oxo-dGuo/dGuo and tail DNA medians remained in the normal range after chemotherapy. Serum vitamin C concentrations decreased significantly after chemotherapy, while other vitamins and carotenoid concentrations were not significantly different.

Vitamins A and E, lutein and zeaxanthin were lower in the patient population than in healthy controls—both before and after chemotherapy. Per cent tail DNA was significantly correlated to vitamin C blood concentration, both before and after chemotherapy ($\rho = -0.517$, $p = 0.023$).

Discussion

To our knowledge, no previous study has compared the responses of comet assay and 8-oxo-dGuo/dGuo in anthracycline-treated patients. While the comet

Table II. Laboratory normal ranges for markers of DNA damage, serum vitamins and carotenoids ($n = 15$).

	Lower limit	Higher limit
8-Oxo-dGuo/10^5 dGuo	0.117	0.825
Comet (% tail DNA)	2.08	6.07
Vitamin C (μM)	30.5	92.5
Vitamin A (μM)	**1.51**	**2.79**
Vitamin E (μM)	**23.9**	**52.2**
Lutein (μM)	**0.424**	**1.74**
Zeaxanthin (μM)	**0.107**	**0.373**
β-Cryptoxanthin (μM)	0.111	0.824
Lycopene (μM)	0.172	1.19
α-Carotene (μM)	0.101	1.19
β-Carotene (μM)	0.202	3.15

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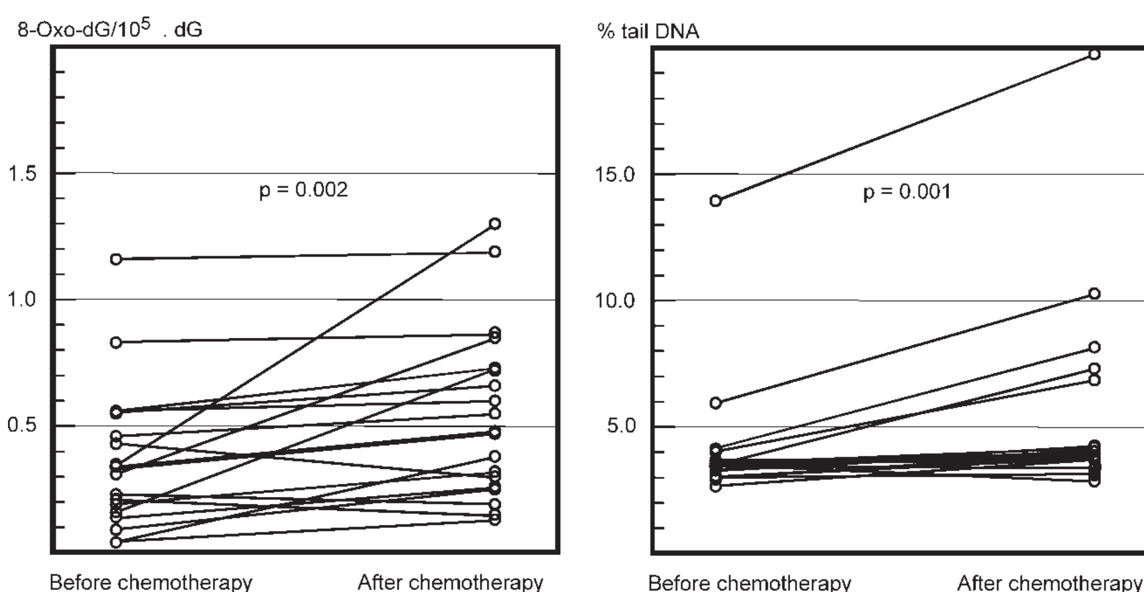

Figure 1. 8-Oxo-dG/dG and comet assays in patients before and after chemotherapy.

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assay detects DNA strand breaks, 8-oxo-dGuo/dGuo measures more specifically the oxidation of DNA guanines. We found that both lesions increased significantly after chemotherapy. However, it should be mentioned that extraction and hydrolysis procedures needed for 8-oxo-dG measurements probably lead to spurious oxidation and an overestimation of actual radical guanine oxidation. Hence, the relative increase on chemotherapy will be underestimated.

As strand breaks can be caused by topoisomerases, their role should be taken into account when comet assays are performed in blood cells. Our results are consistent with the fact that anthracyclines have been shown to induce DNA ladder formation and DNA fragmentation in cardiomyocytes [7]. Indeed, 8-oxo-dGuo formation and strand breaks could be the initial events of morphological changes observed in nuclei; alterations to DNA could be too severe for cardiomyocytes to repair them, and cells then become apoptotic.

The low concentrations of vitamins A and E, and the significant decrease in vitamin C concentrations raises the question of the possible benefit from supplementation for these patients. Indeed, vitamin E is known to be a potent antioxidant [33,34], and can fight free radicals that are generated by epirubicin, particularly in cell membranes where this vitamin concentrates. Vitamin C fights $^{\cdot}$OH radicals [35], and acts synergistically with vitamin E as it regenerates tocopherol from the tocopheryl radical which is formed after tocopherol oxidation by free radicals [36]. Free radicals produced by epirubicin also alter vitamin concentrations probably by consuming them. Plasma vitamin C concentrations not only decreased significantly after chemotherapy, but also correlated significantly to comet assays. As suggested by *in vitro* experiments [20,21], supplementation with these two antioxidant vitamins may help patients fighting free radicals and thus could prevent epirubicin-induced cardiotoxicity and neoplasm. Lutein and zeaxanthin were also lower in patients than in controls. These carotenoids fight singlet oxygen [37], which can alter guanosine [38].

We could not find any significant correlation between epirubicin dosages, 8-oxo-dGuo, comet assays and vitamins, either before or after chemotherapy. Indeed, responses to the oxidative stress and drug action depend not only on plasma vitamin concentrations but also globally on body defences against such attacks. These defences include thiol compounds, proteins, uric acid, some metals and enzymatic systems and each can vary independently from one patient to the other.

In a previous article [39], we showed that urinary 5-hydroxymethyluraci, and TBARs increased significantly, while vitamins A and E decreased after doxorubicin infusion. In the present paper we show that cellular DNA is altered by a less aggressive anthracycline thus inducing a mutagenic alteration of DNA and causing strand breaks. In this study, the decreases in vitamins A and E concentrations were not significant even though they were in our first study. This may be explained by a lower aggressiveness of epirubicin compared to doxorubicin as described elsewhere [40]. In a second study [41], we measured urinary 8-oxo-dGuo and 5-hydroxymethyluracil in adriamycin-treated patients and found no increase in 8-oxo-dGuo. This apparent discrepancy with the present results is not surprising, since repair of 8-oxo-dGuo in DNA (and its elimination in urine) is relatively slow.

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Recently, Doroshow and colleagues measured several doxorubicin-induced lesions by GC-MS [42], and found no increase in 8-oxo-deoxyguanine. Unfortunately, these authors used azathymine as internal standard, while at such levels of concentration GC-MS accuracy requires internal standards labelled with stable isotopes. The chemical DNA hydrolysis [43,44] and derivation processes that are needed before GC-MS measurement are known to oxidise guanine moieties [45,46].

References

[1] Keizer HG, Pinedo HM, Schuurhuis GJ, Joenhe H. Doxorubicin (Adriamycin), a critical review of free radical-dependent mechanisms of cytotoxicity. Pharmacol Therapeut 1990;47:219–231.

[2] Zunino F, Capranico G. DNA topoisomerase II as the primary target of anti-tumor anthracyclines. Anticancer Drug Des 1990;5(4):307–317, Nov.

[3] Hortobagyi GN. Anthracyclines in the treatment of cancer. An overview. Drugs 1997;54(Suppl 4):1–7.

[4] Binaschi M, Bigioni M, Cipollone A, Rossi C, Goso C, Maggi CA, Capranico G, Animati F. Anthracyclines: Selected new developments. Curr Med Chem Anti-Cancer Agents 2001;1(2):113–130, Aug.

[5] Sinha BK, Trush MA, Kennedy KA, Mimnaugh EG. Enzymatic activation and binding of adriamycin to nuclear DNA. Cancer Res 1984;44:2892–2896.

[6] Mizutani H, Oikawa S, Hiraku Y, Murata M, Kojima M, Kawanishi S. Distinct mechanisms of site-specific oxidative DNA damage by doxorubicin in the presence of copper(II) and NADPH-cytochrome P450 reductase. Cancer Sci 2003;94(8):686–691, Aug.

[7] Severs NJ, Twist VW, Powell T. Acute effects of Adriamycin on the macromolecular organization of the cardiac muscle cell plasma membrane. Cardioscience 1991;2/1:35–45.

[8] Keefe DL. Anthracycline-induced cardiomyopathy. Semin Oncol 2001;28(4 Suppl 12):2–7, Aug.

[9] Dziegiel P, Surowiak P, Zabel M, Folia. Correlation of histopathological and biochemical appraisal of anthracycline-induced myocardium damage. Histochem Cytobiol 2002;40(2):127–128.

[10] Kelso EJ, Geraghty RF, McDermott BJ, Cameron CH, Nicholls DP, Silke B. Characterisation of a cellular model of cardiomyopathy in the rabbit produced by chronic administration of the anthracycline, epirubicin. J Mol Cell Cardiol 1997;29(12):3385–3397, Dec.

[11] Nelson MA, Frishman WH, Seiter K, Keefe D, Dutcher J. Cardiovascular considerations with anthracycline use in patients with cancer. Heart Dis 2001;3(3):157–168, May–Jun.

[12] Matesich SM, Shapiro CL. Second cancers after breast cancer treatment. Semin Oncol 2003;30(6):740–748, Dec.

[13] Richard D, Hollender P, Chenais B. Involvement of reactive oxygen species in aclarubicin-induced differentiation and invasiveness of HL-60 leukemia cells. Int J Oncol 2002;21(2):393–399, Aug.

[14] Schimmel KJ, Richel DJ, vandenBrink RB, Guchelaar HJ. Cardiotoxicity of cytotoxic drugs. Cancer Treat Rev 2004;30(2):181–191, Apr.

[15] Hasinoff BB, Schnabl KL, Marusak RA, Patel D, Huebner E. Dexrazoxane (ICRF-187) protects cardiac myocytes against doxorubicin by preventing damage to mitochondria. Cardiovasc Toxicol 2003;3(2):89–99.

[16] Pearlman M, Jendiroba D, Pagliaro L, Keyhani A, Liu B, Freireich EJ. Dexrazoxane in combination with anthracyclines lead to a synergistic cytotoxic response in acute myelogenous leukemia cell lines. Leuk Res 2003;27(7):617–626, Jul.

[17] Robert J. Epirubicin. Clinical pharmacology and dose–effect relationship. Drugs 1993;45(Suppl 2):20–30.

[18] Liu FT, Kelsey SM, Newland AC, Jia L. Generation of reactive oxygen species is not involved in idarubicin-induced apoptosis in human leukaemic cells. Br J Haematol 2001; 115(4):817–825, Dec.

[19] Kelso EJ, Geraghty RF, McDermott BJ, Cameron CH, Nicholls DP, Silke B. Characterisation of a cellular model of cardiomyopathy, in the rabbit, produced by chronic administration of the anthracycline, epirubicin. J Mol Cell Cardiol 1997;29(12):3385–3397, Dec.

[20] DeAtley SM, Aksenov MY, Aksenova MV, Harris B, Hadley R, Harper CP, Carney JM, Butterfield DA. Antioxidants protect against reactive oxygen species associated with adriamycin-treated cardiomyocytes. Cancer Lett 1999;136(1):41–46, Feb 8.

[21] Kumar D, Kirshenbaum L, Li T, Danelisen I, Singal P. Apoptosis in isolated adult cardiomyocytes exposed to adriamycin. Ann N Y Acad Sci 1999;874:156–168, Jun 30.

[22] Loft S, Fisher-Nielsen A, Jeding IB, Vistisen K. 8-Hydroxydeoxyguanosine as a urinary biomarker of oxidative damage. J Toxicol Env Health 1993;40:391–404.

[23] Epe B. Genotoxicity of singlet oxygen. Chem Biol Interact 1991;80:239–260.

[24] Ravanat JL, DiMascio P, Martinez GR, Medeiros MH. Singlet oxygen induces oxidation of cellular DNA. J Biol Chem 2001;276(8):40601–40604, Feb 23.

[25] Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb A. 8-Hydroxyguanine, an abundant form of oxidative DNA damage, cause G-T and A-C substitutions. J Biol Chem 1992;264:166–172.

[26] Jaloszynski P, Masutani C, Hanaoka F, Perez AB, Nishimura S. 8-Hydroxyguanine in a mutational hotspot of the c-Ha-ras gene causes misreplication, ‘action-at-a-distance’ mutagenesis and inhibition of replication. Nucleic Acids Res 2003;31(21):6085–6095, Nov 1.

[27] Faure H, Mousseau M, Cadet J, Guimier C, Tripier M, Hida H, Favier A. Urine 8-oxo-7,8-deoxyguanosine vs. 5-(Hydroxymethyl) uracil as DNA oxidation marker in Adriamycin-treated patients. Free Radic Res 1998;28:377–382.

[28] Hininger I, Chollat-Namy A, Sauvaigo S, Osman M, Faure H, Cadet J, Favier A, Roussel A-M. Assessment of DNA damage by comet assay on frozen total blood: Method and evaluation in smokers and non-smokers. Mutat Res 2004;558:75–80.

[29] Steghens J-P, vanKappel AL, Riboli E, Collombel C. Simultaneous measurement of seven carotenoids, retinol and alpha-tocopherol in serum by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1997;694(1):71–81, Jun 20.

[30] Speek AJ, Schrijver J, Schreurs WH. Fluorometric determination of total vitamin C in whole blood by high-performance liquid chromatography with pre-column derivatization. J Chromatogr 1984;305(1):53–60, Jan 13.

[31] ESCODD Comparative analysis of baseline 8-oxo-7,8-dihydroguanine in mammalian cell DNA by different methods in different laboratories: An approach to consensus. Carcinogenesis 2002;23/12:2129–2133.

[32] Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175(1):184–191, Mar.

[33] Bender DA. Nutritional biochemistry of the vitamins. Nutritional biochemistry of the vitamins. Cambridge, Melbourne, Madrid, Cape Town: Cambridge University Press; 2003., eds.

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[34] Kamal-Eldin A, Appelqvist LA. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 1996;31:671–701.

[35] Frei B, Forte TM, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci 1989;86:6377–6381, USA.

[36] Halpner AD, Handelman GJ, Belmont CA, Blumberg JM. Protection by vitamin C of oxidant-induced loss of vitamin E in rat hepatocytes. J Nutr Biochem 1998;9:355–359.

[37] Di Mascio P, Sundquist AR, Devasagayam TA, Sies H. Assay of lycopene and other carotenoids as singlet oxygen quenchers. Methods Enzymol 1992;213:429–432.

[38] Ravanat JL, Martinez GR, Medeiros MH, DiMascio P, Cadet J. Mechanistic aspects of the oxidation of DNA constituents mediated by singlet molecular oxygen. Arch Biochem Biophys 2004;423(1):23–30, Mar 1.

[39] Faure H, Coudray C, Mousseau M, Ducros V, Douki T, Bianchini F, Cadet J, Favier A. 5-Hydroxymethyluracil excretion, plasma TBARs and plasma antioxidant vitamins in adriamycin-treated patients. Free Radic Biol Med 1996;20(7):979–983.

[40] Kaklamani VG, Gradishar WJ. Epirubicin versus doxorubicin: Which is the anthracycline of choice for the treatment of breast cancer? Clin Breast Cancer 2003;(4 Suppl 1):S26–S33, Apr.

[41] Faure H, Mousseau M, Cadet J, Guimier C, Tripier M, Hida H, Favier A. Urine 8-oxo-7, 8-dihydro-2′-deoxyganosine vs. 5-hydroxymethyluracil as DNA oxidation marker in adriamycin-treated patients. Free Radic Res 1998; 28:377–382.

[42] Doroshow JH, Synold TW, Somlo G, Akman SA, Gajewski E. Oxidative DNA base modifications in peripheral blood mononuclear cells of patients treated with high-dose infusional doxorubicin. Blood 2000;197(9):2839–2845, May.

[43] Cadet J, Douki T, Ravanat JL. Artifacts associated with the measurement of oxidised DNA bases. Environ Health Perspect 1997;105:1033–1039.

[44] Cadet J, d'Ham C, Douki T, Pouget J, Ravanat JL, Sauvaigo S. Facts and artefacts in the measurement of oxidative base damage to DNA. Free Radic Res 1998;29:541–550.

[45] Ravanat JL, Turesky RJ, Gremaud E, Trudel LJ, Stadler RH. Determination of 8-oxoguanine in DNA by gas chromatography–mass spectrometry and HPLC–electrochemical detection: Overestimation of the background level of the oxidised base by the gas chromatography–mass spectrometry assay. Chem Res Toxicol 1995;8(8):1039–1045, Dec.

[46] Ravanat JL, Douki T, Turesky RJ, Cadet J. Measurement of oxidised bases in DNA. Comparison between HPLC–EC and GC–MS assays. J Chim Phys 1997;94:306–312.

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