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CLASTOGENIC AND MUTAGENIC ACTIONS OF ACTIVE SPECIES GENERATED IN THE 6-HYDROXYDOPAMINE/OXYGEN REACTION: EFFECTS OF SCAVENGERS OF ACTIVE OXYGEN, IRON, AND METAL CHELATING AGENTS

P. GEE[1], R.H.C. SAN[2], A.J. DAVISON[3] and H.F. STICH

Environmental Carcinogenesis Unit, B.C. Cancer Research Center, 601 West 10th Ave., Vancouver, B.C. V5Z 1L3 Canada and Bioenergetics Research Laboratory, Faculty of Applied Sciences, Simon Fraser University, Burnaby, B.C. V5A 1S6 Canada. Electronic Mail: adavison@sfu.ca Fax 604-291-3040

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A pro-oxidant triphenol, 6-hydroxydopamine (6-OHDA), induced mutations in the *Salmonella typhimurium* TA104 tester strain (over the concentration range to 800 μM), and induced chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells at lower concentrations (up to 90 μM). It was however only marginally mutagenic (up to cytotoxic levels of 200 μM) in the TA102 tester strain. Clastogenicity in the more sensitive CHO cell assay was mediated by activated oxygen. Superoxide dismutase decreased the incidence of chromosomal aberrations by 60% and catalase (or superoxide dismutase plus catalase) decreased the incidence to control levels. The clastogenicity of 6-OHDA was dependent upon unsequestered transition metal ions, since addition of EDTA plus desferrioxamine decreased chromosomal aberrations by 90%. The simplest explanation of the data is that genotoxicity is mediated by active species generated in a Fenton-type reaction between 6-OHDA and H_2O_2 catalyzed by traces of metals in the medium.

KEY WORDS: genotoxicity, clastogenicity, mutagenicity, 6-hydroxydopamine, superoxide dismutase, catalase, oxygen, radicals, TA104, TA102, CHO cells.

INTRODUCTION

Di- and triphenols include carcinogens, tumor promoters and sometimes anti-genotoxins.[1,2,3] They are effective in systems which range from animal models through cultured mammalian and bacterial cells. Thus, gallic acid,[4,5] caffeic acid,[6] dopamine,[7] and species resulting from redox cycling of quinones,[8] are reportedly mutagenic or clastogenic in cultured cells. Their genotoxic activities are usually attributed to oxygen-derived active species.[9,10] Transition metal ions contribute to oxidant-induced genotoxicity in a variety of test systems.[11,12] Oxygen-derived active species participate in many kinds of genotoxicity, but the extent to which individual active species

[1]Current address: Dept. of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA 94720, USA.
[2]Current Address: Microbiological Associates, 9900 Blackwill Rd., Rockville, MD 20850, USA.
[3]To whom correspondence should be addressed.

participate in the various models for mutagenesis, carcinogenesis and tumor promotion is far from clear.[11]

The genotoxicities of phenolics are not merely of academic interest. Catechols have been reported to be the major co-carcinogens in tobacco smoke.[13] Secondary activation of benzene's phenol metabolites in the bone marrow reportedly mediates benzene's myelotoxic and carcinogenic effects.[14,15,16] Polyphenol-derived active species participate in the carcinogenicity of benzene, tobacco smoke, and tumor promotion by cellular and xenobiotic pro-oxidants.

Two distinct actions of triphenols can induce aneuploidy. First, triphenols can induce scission of microtubules.[17] When this occurs during mitosis, the consequence is aneuploidy in which cytoplasmic micronuclei consist of whole (kinetochore positive) chromosomes.[18] Alternatively, DNA scission[18,19] will lead to cytoplasmic micronuclei consisting of kinetochore negative chromosomal fragments. Active species from triphenol/oxygen interactions reportedly participate in both these mechanisms.[18]

Among benzene metabolites, diphenols and triphenols have been compared in a variety of test systems. 1,2,4-Benzenetriol produced DNA breaks in cultured mammalian cells with an ED_{50} of approximately 55.0 μM.[20,21] In general 1,2,4-triphenols are more cytotoxic[22] and more reactive toward O_2 than diphenols or 1,2,3, triphenols.[23] Mutations to 6-thioguanine resistance were induced by 1,2,4-trihydroxybenzene, or catechol.[24] When sister chromatid exchange, frequencies of micronucleated cells, and mutagenicity were compared, 1,2,4-trihydroxybenzene was overall more potent than hydroquinone, catechol, or phenol. Among catecholamines only the weakly pro-oxidant diphenols have been tested.[7,8] In the current study, therefore, we explore the genotoxicity of a triphenolic amine, 6-hydroxydopamine (6-OHDA or 2,4,5-trihydroxy phenylethylamine).

We selected 6-OHDA as a potential pro-oxidant genotoxin because it autoxidizes rapidly under physiological conditions to generate active species of both oxygen and the quinonoid reaction intermediates. These reactive intermediates include: $O_2^{\cdot-}$, H_2O_2, hydroxyl radicals, singlet state oxygen, and a semiquinone.[25–28] We report: (i) the ability of these intermediates to induce mutations in *Salmonella typhimurium* TA102 and TA104, and chromosomal aberrations in Chinese hamster ovary (CHO) cells, and (ii) protection by enzymic scavengers, and metal chelating agents. While the results meet general criteria for a good model system to test oxidative genotoxicity, some unexpected findings reflect the complex responses of living cells.

METHODS AND MATERIALS

6-Hydroxydopamine (6-OHDA), superoxide dismutase (SOD, from bovine blood, 2800–2900 U/mg) and mitomycin C were purchased from the Sigma Chemical Co. (St Louis, MO). N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Catalase (fungal) was from Cal-Biochem-Behring (88 823 U/mg). NaCl, KCl, sodium and potassium phosphate salts were obtained from Amercan Scientific and Chemical Co. (Seattle, WA) and Matheson Coleman and Bell Manufacturing Chemists (Norwood, OH). $FeCl_3$, $MgSO_4$, ethylenediaminetetraacetic acid (EDTA, disodium salt) and citric acid were purchased from Fisher Scientific Company. Desferrioxamine (Desferal mesylate) was a gift from CIBA Pharmaceutical Co. (Summit, NJ). D-glucose was purchased from BDH (Toronto, Ont.). L-histidine was obtained from N.R.C. General Biochemicals

(Chagrin Falls, OH). Nutrient Broth was obtained from Oxoid Ltd. (Basingstoke, Hants., England). All reagents were of the highest purity commercially available at the time of purchase, and were used without further purification.

Anaerobic preparation of 6-OHDA was accomplished as follows. Deionized distilled buffer was repeatedly flushed with high purity nitrogen (Linde, Union Carbide Canada Ltd.; scrubbed with sodium sulfite) and then evacuated using a Virtis vacuum evacuator. Trapped air in the 6-OHDA powder was gently removed under vacuum and replaced with nitrogen, during dissolution. The vial was sealed under a slight positive pressure of nitrogen to minimize entry of oxygen as aliquots were withdrawn.[29] *Salmonella typhimurium* tester strains TA102 and TA104 were provided by Dr. B.N. Ames (Biochemistry Department, University of California, Berkeley). The standard histidine reversion assay for mutagenicity as described by Maron and Ames[30] was used with minor modifications. Fresh overnight cultures of bacteria (0.1 ml of 1–2 $\times$ 10^9 cells/ml) and phosphate buffered saline (in compensating volumes) were added to melted top agar containing 0.5 mM histidine. Aliquots of 6-OHDA were added to other test reagents, to give a total volume of 2.5 ml. After vortexing, the mixtures were poured on 2% glucose, Vogel-Bonner agar plates. These were incubated at 37°C for 48 h. Three counts of revertant colonies were made on each plate using an automatic colony counter (Artek Systems Corp., Farmingdale, N.Y. Model 880) and the averages of triplicate plates were taken as the value for a given experiment. The points plotted represent these values averaged over a minimum of 5 sets of experiments. Mitomycin C was used as a positive control for mutagenicity testing.

The test for induction of chromosomal aberrations in Chinese hamster ovary (CHO) cells was a modification of the procedure described by Stich and San.[31] CHO cells were cultured in minimal essential medium (MEM) supplemented with 10% fetal bovine serum (from Gibco Laboratories, Bethesda, MD) on 22 mm^2 coverslips in 3.5 cm plastic petri dishes until 40-60% confluent. Following a 3 h exposure to 6-OHDA and/or other reagents at 37°C, the cells were washed with MEM, and incubated at 37°C in fresh MEM completed with 10% fetal bovine serum. After 16 h, cells were arrested at metaphase by the addition of colchicine to a final concentration of 10 μg/ml. After 4 h the cells were treated with 1% sodium citrate for 20 min, fixed in acetic acid/methanol (1 : 3) for 20 min and air dried before staining with 2% orcein and mounting on slides with permount. For each cell culture, 100 metaphases were analyzed for chromatid breaks and chromatid exchanges (as described in Reference 31). MNNG was used as a positive control in the CHO genotoxicity testing.

RESULTS

Mutagenicity of 6-OHDA in Salmonella typhimurium

When assayed over concentrations from 0 to 800 μM, 6-OHDA caused a dose-related increase in revertant colony count in *Salmonella typhimurium* strain TA104 (Figure 1). At 800 μM, 6-OHDA caused a 5-fold increase in colony count above untreated controls while the response of TA102 was much less. Moreover in this strain the progressive decrease in revertant colony count at dose levels above 200 μM suggests a toxic action of 6-OHDA. Although we were eventually able to obtain five qualitatively similar replications of these dose response curves, we should caution other

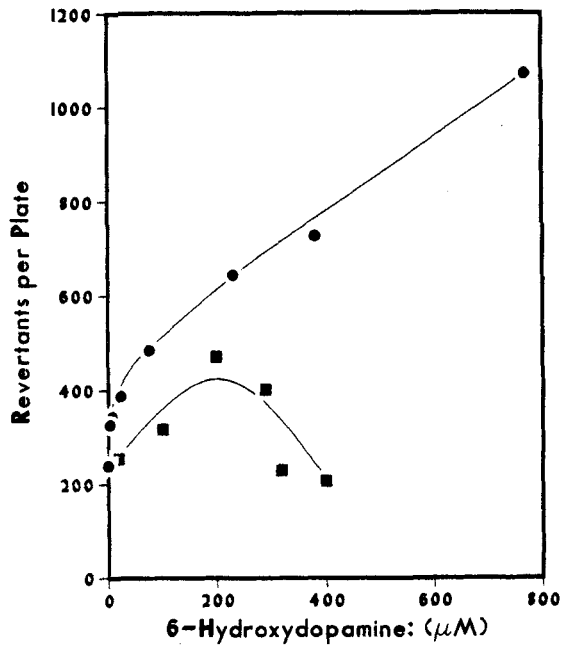

FIGURE 1 Effect of 6-hydroxydopamine on the histidine reversion of *Salmonella typhimurium* strains TA102 and TA104. Aliquots of bacteria plus 6-OHDA were plated on 2% glucose agar. The Y axis shows the mean of three colony counts of each of three separate plates after 48 h incubation at 37°C. Strains TA102 are shown as squares and TA104 are circles. The computer-fitted lines are generic (spline) curves intended only to approximate the data.

workers that both the TA102 and TA104 strains were quite unstable in our experiments.

Clastogenicity of 6-OHDA in CHO Cells

Exposure of CHO cells to a 6-OHDA (0 to 80 μM for 3 h) resulted in a dose-related increase in chromosomal breaks and exchanges (Figure 2). Each point was averaged over triplicate slides, and four sets of experiments gave qualitatively similar results. The sensitivity of the CHO cells was an order of magnitude greater than that of the TA104 tester strain. Only at the highest concentrations did 6-OHDA inhibit mitosis (indicative of toxicity). Because of the greater sensitivity and reliability of the CHO cell test, we used the CHO cells as the test system of choice in the ensuing studies of the effects of scavengers, metal ions, metal chelating agents and antioxidants, despite the increased time required for the assays.

Effects of Scavengers of Active Oxygen

As can be seen in Figure 2, the chromosomal damaging effect was eliminated or dramatically decreased by the addition of superoxide dismutase, catalase, or both together. Catalase alone or in combination with superoxide dismutase afforded virtually complete protection, no aberrations being detected at 6-OHDA concentrations

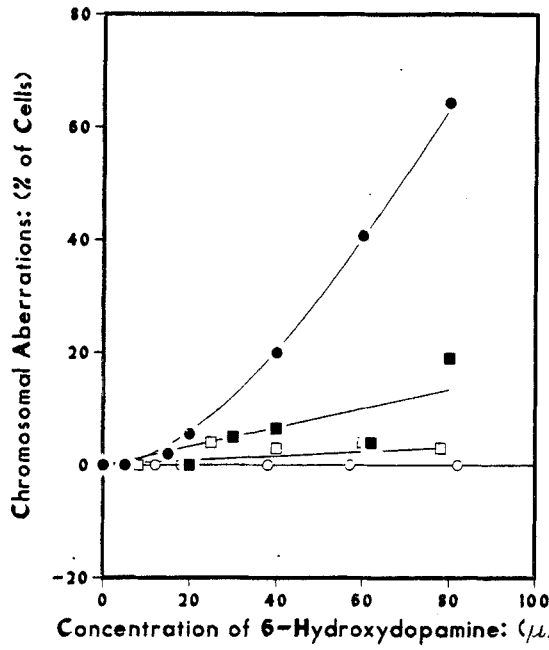

FIGURE 2 Chromosomal aberrations induced by 6-hydroxydopamine in CHO cells: Effects of scavengers of active oxygen. Cells were exposed to aliquots of 6-OHDA for 3 h at 37°C, pH 7.4. Scavengers were added prior to addition of 6-OHDA. The Y axis represents chromosomal aberrations per 100 cells. The topmost line is a computer generated generic (spline) curve fitted to the data for 6-OHDA without added scavengers, represented by filled circles. The intermediate curves show 6-OHDA + SOD (50 U/ml, filled squares); 6-OHDA + catalase (50 U/ml, open squares); The data along the Y = 0 line are for 6-OHDA with SOD + catalase simultaneously present (open circles).

up to 80 μM. Superoxide dismutase also inhibited the clastogenic activity of 6-OHDA, but only by 60%. Our attempts to determine the effects of ascorbate on the genotoxicity of 6-OHDA were inconclusive, since the addition of ascorbate (to 0.1 mM) increased cytotoxic effects to the point where mitotic inhibition was apparent at 30 μM 6-OHDA. However an increase in the genotoxicity of 6-OHDA in the presence of ascorbate is suggested by the occurrence of a few (5%) chromosomal aberrations at 6-OHDA concentrations as low as 5 μM. Since there were insufficient surviving cells to allow statistical confidence, the data were not plotted.

Effects of Metals and Metal Chelating Agents

Figure 3 reveals that addition of EDTA plus desferrioxamine decreases 6-OHDA-induced chromosomal aberrations by 90%. To follow up the role of iron as implied by the protection afforded by desferrioxamine, we examined the effects of added iron salts. Since we were specifically interested in effects dependent on 6-OHDA-mediated redox cycling, only the ferric form of iron was used (the ferrous form could generate active oxygen directly). Thus any redox effects were dependent on reducing equivalents provided by 6-OHDA-mediated redox cycling of stoichiometric amounts of iron. Surprisingly (in view of the inhibition by metal chelating agents), the addition

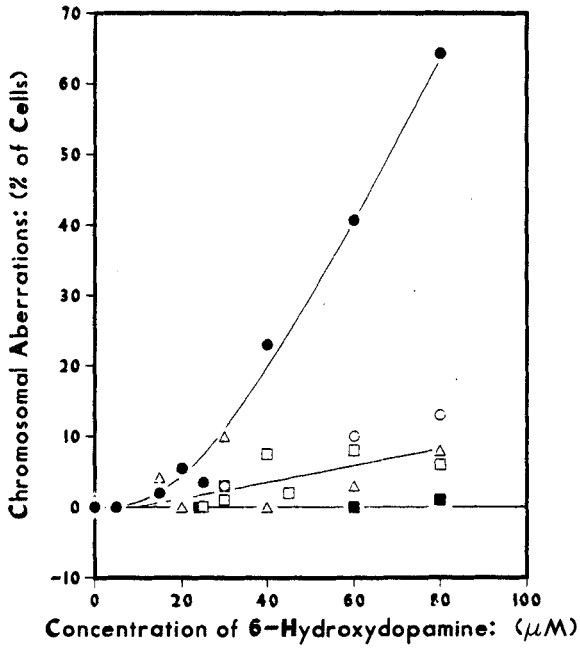

FIGURE 3 Chromosomal aberrations induced by 6-hydroxydopamine in CHO cells: roles of metal ions and chelating agents. Experimental conditions were states as in Figure 2. The topmost line fits the data for 6-OHDA alone, represented by filled circles. The intermediate line does not distinguish between three sets of overlapping data: 6-OHDA + ferric chloride at 0.1 mM (open squares); 6-OHDA + 1.0 mM EDTA + 0.5 mM desferrioxamine (open triangles); 6-OHDA + 1.0 mM ferric chloride + 1.0 mM EDTA (open circles). The base values along the Y = 0 line are the data for 6-OHDA + SOD (50 U/ml) + catalase (50 U/ml) + 1.0 mM EDTA + 0.5 mM desferrioxamine shown as filled squares. The points in this last set of were indistinguishable from control levels (no 6-OHDA added).

of Fe^{3+} (to 100 μM) *protected.* Thus Fe^{3+} decreased the frequency of chromosomal aberrations and increased the threshold to 6-OHDA, affording a level of protection comparable to that provided by superoxide dismutase. In an effort to demonstrate a toxic effect of added iron salts, EDTA was added together the iron, (EDTA is reported to increase the participation of iron in Fenton-type reactions). However the protective effect of iron persisted in the presence of a ten-fold molar excess of EDTA. In contrast to the effects on the above measures of *genotoxicity*, iron or iron plus EDTA did not alter *cytotoxicty* of 6-OHDA as measured by mitotic inhibition at any 6-OHDA concentration (data not shown).

DISCUSSION

Genotoxicity of 6-OHDA

From the current data 6-OHDA meets the criteria for a useful test reagent for oxygen mediated genotoxicity. Mammalian cells were superior to *Salmonella typhimurium* tester strains as probes for this kind of genotoxicity. These findings agree with results of Moldeus *et al.*[7] Reflecting its greater reactivity toward oxygen, 6-OHDA in the

current studies produced more chromosomal aberrations and was effective at concentrations more than two orders of magnitude lower than dopamine.[7] The clastogenic actions of dopamine were inhibited by superoxide dismutase,[7] as was the case with 6-OHDA in the current studies.

Dopamine was not mutagenic in the *Salmonella typhimurium* tester strains examined in previous studies: TA1535, TA100, TA1538, TA98, TA1537.[7] These findings agree with the current marginal mutagenicity of 6-OHDA toward the TA102 tester strain. However we were able to show substantial mutagenicity using the TA104 tester strain, in agreement with the studies in the same tester strain using redox cycled quinones as a free radical source.[8] The relative insensitivity of the TA102 strain to mutagenesis by 6-OHDA was unexpected, in that the 6-OHDA/O_2 system has been repeatedly shown to generate $O_2^{\cdot-}$, H_2O_2 and $^{\cdot}OH$, to which the TA102 strain is reportedly to be sensitive.[32]

The threshold dosage of 6-OHDA below which no genotoxicity is seen (15 μM) suggests that the amounts of free radicals generated by concentrations of 6-OHDA below 15 μM can be absorbed by portions of the cell (such as membrances and antioxidant defenses) in which damage does not lead to chromosomal aberrations. There is ample precedent for such threshold effects in a variety of other free radical mediated processes.[33] In the *Salmonella* mutagenicity test, such a small threshold dose was detectable in comparison with the higher concentrations of 6-OHDA required.

Effects of Scavengers of Active Oxygen

The complete inhibition of *genotoxicity* by catalase and partial inhibition by superoxide dismutase parallel the effects of these scavengers on the *cytotoxicity* of 6-OHDA seen in the current study and in earlier studies.[22] Clearly, 6-OHDA alone is not an important clastogenic agent (otherwise it would not be inhibited by scavengers of its oxidation products). On this basis, the almost total inhibition by catalase or desferrioxamine may reflect a major contribution of ferrous iron and H_2O_2 interacting via Fenton-type reactions.

Either 6-OHDA or superoxide can serve as the Fenton reductant. However, despite the substantial inhibition by superoxide dismutase, $O_2^{\cdot-}$ is not a strong candidate as an important Fenton electron donor. This is because 6-OHDA which itself reduces H_2O_2[29] is present at much higher concentrations than $O_2^{\cdot-}$. Why then does superoxide dismutase inhibit? One suggestion is that *in vitro*, superoxide dismutase decreases the rate of reaction of 6-OHDA with O_2, by over 90%.[26,27] Thus despite the fact that H_2O_2 is a product of the action of superoxide dismutase, superoxide dismutase dramatically *decreases* the production of H_2O_2.[28] The 6-OHDA/O_2 reaction depends on $O_2^{\cdot-}$ and semiquinone radicals for propagation. Superoxide dismutase in the presence of oxygen accelerates removal of both $O_2^{\cdot-}$ and semiquinone radicals.[23]

The conclusion of Chesis *et al.*[8] that superoxide is the primary mediator of the genotoxic actions of the quinone/quinol redox systems studied is not directly consistent with the relatively less important role of superoxide (in comparison with H_2O_2) in the current system. In the light of propagation of radical production by superoxide, protection by superoxide dismutase does not provide unequivocal evidence of a role for $O_2^{\cdot-}$ as the primary genotoxic agent. In considering the complete protection against the genotoxicity of 6-OHDA by the simultaneous presence of EDTA, desferrioxamine, superoxide dismutase and catalase it must be considered that this combination of scavengers completely blocks the reaction of 6-OHDA with oxygen *in vitro*.[27]

Effects of Metals and Metal Chelating Agents

Since iron or iron + EDTA accelerate the autoxidation of 6-OHDA,[26,28] the protective action of iron is explicable on the basis that the iron accelerates removal of the 6-OHDA (resulting in a briefer, albeit more intense, pulse of free radicals). A similar explanation is given for protection by transition metal ions (Mn, Cu, Fe) against the mutagenic actions of quercetin in TA98 tester strain.[5] Alternatively, at the highest concentrations, iron might act primarily as a *scavenger* of reactive species, decomposing either $O_2^{\cdot-}$ or H_2O_2 before they could enter the cell and damage the DNA. Against this are the protective actions of EDTA which promotes iron-catalyzed oxidations and Fenton reactions.[34] Further experiments are needed to assess the roles of other metals, hydroxyl radicals, and also the dose dependence with respect to iron in the concentration range between the stimulatory and inhibitory effects, i.e., between 10 μM and 100 μM.

CONCLUSION

In summary then, intermediates in the autoxidation of 6-OHDA are genotoxic on two criteria: mutagenesis in the *Salmonella typhimurium* test, and chromosomal aberrations in CHO cells. Of these two systems the CHO cell line was more sensitive by an order of magnitude. 6-OHDA is a more effective genotoxin than dopamine, but less effective than quinones in the presence of reducing systems which allow redox cycling of the quinone.[8] Mediation by H_2O_2 and transition metal ions in a Fenton-type mechanism is crucial to the genotoxicity, but raising the concentration of iron to 100 μM inexplicably protected.

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References

1. E.P. Cronkite, J.E. Bullis, T. Inoue and R.T. Drew (1984) Benzene inhalation produces leukemia in mice. *Toxicology and Applied Pharmacology*, **75**, 358–361.
2. M.J. Laughton, B. Halliwell, P.J. Evans and J.R.S. Hoult (1989) Antioxidant and pro-oxidant actions of the plant pheolics quercetin, gossypol and myricetin. *Biochemical Pharmacology*, **17**, 2859-2865.
3. P.D. Josephy, H.L. Lord and V.A. Snieckus (1990) Inhibition of benzo[a] pyrene dihydrodiol epoxide mutagenicity by synthetic analogues of ellagic acid. *Mutation Research*, **242**, 143–149.
4. H.F. Stich, M. Rosin, C.H. Wu and W.D. Powrie (1981) The action of transition metals on the genotoxicity of simple phenols. *Cancer Letters*, **14**, 251–260.
5. J.F. Hatcher and G.T. Bryan (1985) Factors affecting the mutagenic activity of quercetin for Salmonella typhimurium TA98: metal ions, antioxidants and pH. *Mutation Research*, **148**, 13–23.
6. A.F. Hanham, B.P. Dunn and H.F. Stich (1983) Clastogenic activity of caffeic acid and its relationship to hydrogen peroxide generated during autoxidation. *Mutation Research*, **116**, 333–339.
7. P. Moldeus, M. Nordenskjold, G. Bolcsfoldi, A. Eiche, U. Haglund and B. Lambert (1983) Genetic toxicity of dopamine. *Mutation Research*, **124**, 9–24.
8. P.L. Chesis, D.E. Levin, M.T. Smith, L. Ernster and B.N. Ames (1984) Mutagenicity of quinones: pathways of metabolic activation and detoxification. *Proceedings of the National Academy of Science USA*, **81**, 1696–1700.

9. M. Rosin (1984) The influence of pH on the convertogenic activity of plant phenolics. *Mutation Research*, **135,** 109–113.
10. B.N. Ames (1983) Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. *Science*, **221,** 1256–1264.
11. B. Halliwell and O.I. Aruoma (1991) DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. *FEBS Letters*, **281,** 9–19.
12. O.I. Aruoma, B. Halliwell, E. Gajewski and M. Dizdaroglu (1991) Copper-ion-dependent damage to the bases in DNA in the presence of hydrogen peroxide. *Biochemical Journal*, **273,** 601–604.
13. S. Hecht, S. Carmella, H. Mori and D. Hoffmann (1981) A study of tobacco carcinogenesis. Role of catechol as a major cocarcinogen in the weakly acidic fraction of smoke condensate. *Journal of the National Cancer Institute*, **66,** 163–169.
14. W.F. Greenlee, E.A. Gross and R.D. Irons (1981) Relationship between benzene toxicity and the disposition of ^{14}C-labeled benzene metabolites in the rat. *Chemico-Biological Interactions*, **33,** 285–299.
15. M.T. Smith, J.W. Yager, K.L. Steinmetz and D.A. Eastmond (1989) Peroxidase-dependent metabolism of benzene's phenolic metabolites and its potential role in benzene toxicity and carcinogenicity. *Environmental Health Perspectives*, **82,** 23–29.
16. J.G. Lewis, W. Steward and D.O. Adams (1988) Role of oxygen radicals in induction of DNA damage by metabolites of benzene. *Cancer Research*, **48,** 4762–4765.
17. A.J. Davison, N.A. Legault and D.W. Steele (1986) Effect of 6-hydroxydopamine on polymerization of tubulin. Protection by superoxide dismutase, catalase, or anaerobic conditions. *Biochemical Pharmacology*, **35,** 1411–1417.
18. J.W. Yager, D.A. Eastmond, M.L. Robertson, W.M. Paradisin and M.T. Smith (1990) Characterization of micronuclei induced in human lymphocytes by benzene metabolites. *Cancer*, **50,** 593–399.
19. S. Kawanishi, S. Inoue and M. Kawanishi (1989) Human DNA damage induced by 1,2,4-benzenetriol, a benzene metabolite. *Cancer Research*, **49,** 164–168.
20. K. Yamada, S. Shirahata, H. Murakami, K. Nishiyama, K. Shinohara and H. Omura (1985) DNA breakage by phenyl compounds. *Agricultural and Biological Chemistry*, **49,** 1423–1428.
21. P. Pellack-Walker and J.L. Blumer (1986) DNA damage in L5178YS cells following exposure to benzene metabolites. *Molecular Pharmacology*, **30,** 42–47.
22. E. Tiffany-Castiglioni, R.P. Saneto, P.H. Proctor and J.R. Perez-Polo (1982) Participation of active oxygen species in 6-hydroxydopamine toxicity to a human neuroblastoma cell line. *Biochemical Pharmacology*, **31,** 181–189.
23. B. Bandy, J. Moon and A.J. Davison (1990) Multiple actions of superoxide dismutase: why can it both inhibit and stimulate reduction of oxygen by hydroquinones? *Free Radical Biology & Medicine*, **9,** 143–148.
24. H. Glatt, R. Padykula, G.A. Berchtold, G. Ludewig, K.L. Platt, J. Klein and F. Oesch (1989) Multiple activation pathways of benzene leading to products with varying genotoxic characteristics. *Environmental Health Perspectives*, **82,** 81–89.
25. G. Cohen and R.E. Heikkila (1974) The generation of hydrogen peroxide, superoxide radical, and hydroxyl radical by 6-hydroxydopamine, dialuric acid, and related cytotoxic agents. *Journal of Biological Chemistry*, **249,** 2447–2452.
26. S.G. Sullivan and A. Stern (1981) Effects of superoxide dismutase and catalase on catalysis of 6-hydroxydopamine and 6-aminodopamine autoxidation by iron and ascorbate. *Biochemical Pharmacology*, **30,** 2279–2285.
27. P. Gee and A.J. Davison (1984) 6-Hydroxydopamine does not reduce molecular oxygen directly, but requires a coreductant. *Archives of Biochemistry and Biophysics*, **231,** 164–169.
28. A.J. Davison and P. Gee (1984) Redox state of cytochrome C in the presence of the 6-hydroxydopamine/oxygen couple: oscillations dependent on the presence of hydrogen peroxide or superoxide. *Archives of Biochemistry and Biophysics*, **233,** 761–771.
29. P. Gee and A.J. Davison (1985) Effects of scavengers of oxygen free radicals on the anaerobic oxidation of 6-hydroxydopamine by $H_2O_2^{\cdot}$. *Biochimica et Biophysica Acta*, **838,** 183–190.
30. D.M. Maron and B.N. Ames (1983) Revised methods for the Salmonella mutagenicity test. *Mutation Research*, **113,** 173–215.
31. R.H.C. San and H.F. Stich (1970) DNA repair and chromatid anomalies in mammalian cells exposed to 4-nitroquinoline N-oxide. *Mutation Research*, **10,** 389–404.
32. D.E. Levin, M. Hollstein M.F. Christman, E.A. Schwiers and B.N. Ames (1982) A new Salmonella tester strain (TA102) with A:T base pairs at the site of mutation detects oxidative mutagens. *Proceedings of the National Academy of Science USA*, **79,** 7445–7449.

33. D.D. Wayner, G.W. Burton, K.U. Ingold, L.R. Barclay and S.J. Locke (1987) The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma. *Biochimica et Biophysica Acta*, **942**, 408–419.
34. B. Halliwell (1978) Superoxide-dependent formation of hydroxyl radicals in the presence of iron chelates: is it a mechanism for hydroxyl radical production in biochemical systems? *FEBS Letters*, **92**, 321–326.

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