ELSEVIER

Toxicology 181–182 (2002) 433–439

TOXICOLOGY

www.elsevier.com/locate/toxicol

Recent advances in the study of epigenetic effects induced by the phycotoxin okadaic acid

Edmond Ekué Creppy [a,*], Adama Traoré [a], Isabelle Baudrimont [a], Marta Cascante [b], Maria-Rosaria Carratú [c]

[a] *Department of Toxicology, Laboratory of Toxicology and Applied Hygiene, University Victor Segalen Bordeaux 2, 146, rue Léo-Saignat, 33076 Bordeaux, France*

[b] *Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain*

[c] *Department of Pharmacology, University of Bari, Bari, Italy*

Abstract

Okadaic acid (OA) is a phycotoxin produced by dinoflagellates. It accumulates in the digestive tracts of shellfish causing diarrhetic shellfish poisoning (DSP) in consumers. OA is a tumour promoter, and an inhibitor of both protein phosphatases and protein synthesis. OA induces DNA adducts, suggesting it may be carcinogenic. Since the Ames test without S_9 was negative, but a mutagenesis test was positive in mammalian cells, the question as to whether its molecular mechanism is genotoxic or epigenetic became unavoidable. Therefore, experiments were performed to search for epigenetic effects, since evidence for DNA-adduct formation using the γ-^{32}P-ATP post-labelling method was not obtained. We found that OA is a potent inducer of lipid peroxidation in human intestinal cells (Caco-2) at low concentrations (0.75–7.5 ng/ml versus IC50 of 15 ng/ml) with increased rates of 8-OH-dG and m^{5}dC formation causing CG to AT transversion mutations and gene deregulation, respectively. The transcription and translation of connexin 43-specific mRNA were inhibited, and ^{3}H-uridine incorporation in RNA was concomitantly increased. Consequently gap junction intracellular communication (GJIC) was inhibited, making possible cellular anarchic proliferation. Higher OA concentrations also disorganized the cellular cytoskeleton, since both actin and tubulin formations were impaired. Our results suggest that OA may induce tumours via an epigenetic mechanism.

Keywords: Phycotoxins; Okadaic acid; Human intestinal Caco-2 cells; Gap junction intracellular communication (GJIC) inhibition; Epigenetic mechanisms of carcinogenesis

1. Introduction

Okadaic acid (OA) was first isolated from the black sponge *Holichondria okadaii* (Tachibana et al., 1981), and later in the dinoflagellate *Prorocentrum lima* (Murakami et al., 1982). OA is the main dinoflagellate toxin in the hepatopancreas of mussels (Amzil et al., 1992; Ciminiello et al.,

* Corresponding author. Tel.: +33-5-5757-1217; fax: +33-5-5698-6685

E-mail address: edmond.creppy@tox.u-bordeaux2.fr (E.E. Creppy).

0300-483X/02/$ - see front matter
PII: S0300-483X(02)00489-4

1999), which are also invariably contaminated by environmental pollutants such as heavy metals (mainly Cd, Hg, Pb) and aluminium (Traoré et al., 1999a). Previous toxicokinetic studies have shown that OA is widely distributed in the bodies of mammals after consumption (Matias et al., 1999a) although the main symptoms are in the intestinal tract, where it is responsible for diarrhetic shellfish poisoning (DSP) (Kumagai et al., 1986; Sueoka and Fujiki, 1997). This phycotoxin is a tumour promoter (Fujiki and Suganuma, 1999), an inducer of lipid peroxidation (Traoré et al., 1999b, 2000; Guzman and Castro, 1991; Matias et al., 1999b) and an inducer of cytotoxicity and apoptosis in cultured mammalian cells (Boe et al., 1991; Lerga et al., 1999; Traoré et al., 1999b, 2000; Berven et al., 2001). Matias and Creppy (1998) showed that at low concentrations, OA significantly increases DNA methylation. Thus, OA may interfere with gene regulation, expression and cellular proliferation and participate in epigenetically-induced tumours by a gap junction intracellular communication (GJIC) inhibition mechanism.

Gap junctions are specialised plasma membrane-associated proteins that form intercellular channels that co-ordinate tissue function by allowing cell to cell passage of ions and small molecules. They are composed of connexins, some of which have been cloned from multiple species. Their sequences have turned out to be fairly well conserved throughout species; e.g. connexin 43 appears to be the most ubiquitous connexin, sharing 97% amino acid identity among mammals (Beyer, 1993). Many tumour promoters have been shown to inhibit GJIC (Upham et al., 1997; Kenne et al., 1994). Inhibition of GJIC between initiated and surrounding normal cells by tumour promoters is believed to be important in the promotion stage of carcinogenesis (Budunova et al., 1996).

Carcinogenesis may be associated with various epigenetic mechanisms, which can alter intra-and intercellular communication and gene expression. These processes affect cytokinetics, i.e. regulation of cell proliferation, differentiation, and apoptosis. These processes lead to a loss of homeostatic control. In addition to epigenetic events such a DNA methylation and histone acetylation, the major mechanisms include: (i) oxidative stress; (ii) changes in concentration of signal molecules; (iii) modulation of cell receptors and metabolizing enzymes; and (iv) interference with intracellular signal transduction pathways (Hofmanova et al., 2000).

One of the most prevalent products of oxygen radical injury in DNA is 8-hydroxyguanosine. Cells must be able to withstand damage by oxygen radicals and possess specific repair mechanisms that correct this oxidative lesion. However, when these defenses are over-saturated, such as under conditions of high oxidative stress, or when repair is inefficient, the miscoding potential of this lesion can result in mutations in the mammalian genome. In addition to causing genetic changes, active oxygen species can lead to epigenetic alterations in DNA methylation, without changing the DNA base sequence. Such changes in DNA methylation patterns can strongly affect the regulation of expression of many genes (Cerda and Weitzman, 1997).

Since OA is a tumour promoter and could be suspected of participation in tumour promotion in the human gut, experiments have been designed using a human colorectal adenocarcinoma cell line, Caco-2, to study the effects of this marine toxin in several epigenetic mechanisms. These mechanisms include: (i) production of oxidative stress and oxidized DNA bases; (ii) production of m5dC; (iii) inhibition of GJIC; (iv) inhibition of the synthesis of connexin 43 with RT-PRC technique and immunohistochemistry; and (v) cytotoxicity. Our results are compared to existing results on tumour promotion by OA.

2. Materials and methods

2.1. Chemicals

OA was obtained from RBI, Research Biochemical International (Natick, USA), [4,5-^{3}H(N)]-uridine (specific activity 22.5 Ci/mmol) was obtained from DU PONT-NEN Products (Boston, USA).

2.2. Cell culture and maintenance

Caco-2 cells were routinely cultured in a humidified 5% CO_2/95% air mixture at 37 °C. Cells were grown in DMEM medium (Sigma, France) supplemented with 10% foetal bovine serum, 8 mM L-glutamine, penicillin (100 IU/ml), and streptomycin (100 μg/ml).

2.3. Cytotoxicity assay

Cytotoxicity was evaluated using the trypan blue dye exclusion test, lactate dehydrogenase (LDH) release (Test kit DG-140-K Sigma, France) MTT oxidation (Test kit MTS-cell-titer 96, Promega, USA) based on the capacity of viable cells to metabolize a tetrazolium colourless salt to a blue formazan in the mitochondria after 72 h of incubation with the toxin or the vehicle.

2.4. Extraction and determination of MDA–TBA-adduct

Lipid peroxidation was measured by quantification of malondihaldehyde (MDA) formed during incubation, as previously described by Matias et al. (1999b) using the thiobarbiturate (TBA)–MDA-adduct formation, followed by HPLC separation and fluorometric detection.

2.5. Cellular [^{3}H]-uridine incorporation

Caco-2 cells were cultured (2×10^5 cells/ml) in microplates, total volume 3 ml/well (Greiner Labortechnik, Germany), for 24 h at 37 °C. OA was dissolved in ethanol, then diluted in culture medium at 10 μl/ml. A range of OA concentrations (3.75–60 ng/ml) were added for a 24 h incubation (only ethanol and medium as control). After this incubation period, 1.0 μCi of [^{3}H]-uridine, was added during a 3 h-pulse, and cells were treated to determine the incorporated radioactivity into viable cells, and compared to controls as previously described by Matias et al. (1996).

2.6. Reverse transcription-polymerase chain reaction (RT-PCR)

RNA was extracted with RNeasy Mini Kit reagents (QIAGEN, Germany) according to the manufacturer's protocol after 24 h of incubation with either OA or vehicle. Reverse transcription of 1 μg of total RNA was performed in a final volume of 24 μl for 1 h at 42 °C, using 30 U of AMV reverse transcriptase (Promega, France), 40 U of RNA inhibitor, 1 mmol/l each of dATP, dGTP, dCTP, and dTTP and 2.5 mmol/l oligo (dT) (Promega, France). The PCR products were analysed on 1.5% agarose gel in 0.5 × Tris borate–EDTA buffer. Gels were stained with ethidium bromide and photographed using Polaroid type 667 film.

2.7. Determination of the rate of methylation and 8-OH-dG

The DNA of treated and control cells was extracted, and subjected to enzymatic treatment for evaluation of the rate of 8-OH-dG formation, by HPLC-electrochemical detection and the rate of m5dC formation by HPLC–UV detection as previously described by Traoré et al. (2000).

2.8. Indirect immunofluorescent staining of gap junctions

Immunostaining of Cx-43-containing gap junctions in Caco-2 cells was performed with a rabbit polyclonal antibody against Cx-43, after 24 h of incubation with either toxin or vehicle. The cells were incubated with anti-Cx43 polyclonal antibody diluted in PBS for 2 h at room temperature (Zymed Laboratories Inc., USA), washed four times with PBS containing 1% BSA and 0.05 Tween 20, and incubated with FITC-conjugated goat *anti*-rabbit IgG (whole molecule) (Sigma Chemical Co., France) diluted 1:200 in the PBS with 1% BSA 0.05% Tween 20 for 1 h at room temperature. The cells were viewed and photographed using an ultima confocal microscope (Meridian Instrument Co., Okemos, MI, USA).

3. Results

3.1. Cytotoxicity of OA

OA decreased the number of viable cells in a concentration-dependent manner (Table 1). For an OA concentration of 15 ng/ml, only 50% of cells were viable. The MTT test showed similar results for mitochondrial activity (Table 1). These results confirm the cytotoxicity of OA, in accordance with results obtained by assessing the release of LDH into culture medium (Table 1).

3.2. Cellular [^3H]-uridine incorporation

OA increased the incorporation of radiolabelled uridine in cells in a concentration-dependent manner, indicating a higher transcription rate (Table 1). The concentration reducing cell viability by 50% induced more than a 100% increase in the rate of uridine incorporation into newly-synthesised total RNA.

3.3. Lipid peroxidation

DNA base modifications were determined in Caco-2 cells. Lipid peroxidation increased by 70% at a concentration of OA of 0.075 ng/ml, and by 120% at 15 ng/ml. At the same time the production of 8-OH-dG increased from 38 to 52 per 10^5 dG and m^5dC increased from 3.5% of (dC+m^5dC) to 6%.

3.4. Reverse transcription-polymerase chain reaction (RT-PCR)

OA (3.75–60 ng/ml) decreased the mRNA levels at concentrations above 15 ng/ml until a total disappearance of Cx-43 gene transcripts occurred after 24 h incubation (results not shown).

3.5. Indirect immunofluorescent staining of gap junction

Membrane-localized gap junctional plaques were readily apparent in control cells. OA inhibited membrane plaques of gap junction from membranes in a dose-dependent manner. This interruption of the gap junction is characterized by numerous pericellular underlines (results not shown).

4. Discussion

In considering possible carcinogenesis mechanisms of OA via epigenetic effects it is necessary to examine the relationship between DNA mutagenic lesions (8-OH-dG), and DNA methylation, and the role played by these changes in the process of carcinogenesis. Many types of DNA damage (oxidative lesions, alkylation of bases and non-base sites, photodinners, etc.) interfere with the ability of mammalian cell DNA to be methylated at CpG dinucleotides by DNA-methyltransferases. This can result in altered patterns in the distribution of 5-methylcytosine (m5dC) residues at CpG sites. Methylation of DNA is an epigenetic change that is nevertheless heritable. It can result in changes in chromatin structure, and it is often accompanied by modified patterns of gene expression. Examples are excessive expression of oncogenes and repression of tumour suppressing genes (Wachsman, 1997).

The relative increase of [^{3}H]-uridine incorporation in cells treated with OA can be explained by several mechanisms. Caco-2 cells may react to OA treatment by inducing the synthesis of mRNA encoding oncoproteins, due to modification of the rate of m5dC in the DNA as shown by Matias and Creppy (1998).

OA-induced inhibition of connexin43 could be due to: (i) a specific decrease of mRNA expression (transcription); (ii) to a post-transcriptional factor such as decrease of mRNA stability; (iii) increased protein degradation; or (iv) combinations of the above. Each of these mechanisms has been reported to occur (Baker et al., 1995; Cesen-Cummings et al., 1998). In the present investigations it is unlikely that increased RNA degradation occurred, since a RNAase inhibitory agent was always in the medium and since degradation does not occur in controls and other cell samples. Moreover, the inhibition of connexin synthesis has been confirmed by immunohistochemistry. It could be that some stress-induced proteins such as

Table 1
Cytotoxicity of OA as evaluated by Trypan blue exclusion test, MTT test, LDH test and synthesis of total RNA measured by ^{3}H-uridine incorporation in Caco-2 cells after 24 h of incubation

	Okadaic acid concentration in culture medium (ng/ml)					
	Control	3.75	7.5	15	30	60
Trypan blue test (no. of viable cells)	201 250±51 821	200 625±40 997	131 875±47 888	106 875±41 951	78 750±9242	53 125±12 141
LDH test (UI/ml released into the culture medium)	5610	5206	5923	7217	11 973	11 876
MTT test (formazan formation relative to untreated controls)	100	100±4.3	86±3.7	46.8±2.2	12.4±0.8	6±0.2
^{3}H-Uridine incorporation in cells (cpm)	2897±297	3187±227	3236±103	3236±120	3553±386	4685±1072

heat shock proteins are induced in Caco-2 cells as previously suggested (Chang et al., 1993). In the present study, OA appears to have inhibited Cx-43 mRNA synthesis in Caco-2 cells. Unexpectedly, ^{3}H-uridine incorporation was increased at the same time, suggesting that the inhibition of connexin 43 mRNA synthesis is a specific effect.

At the same time OA inhibits protein synthesis by hyperphosphorylation of elongation factor EF2 in a very specific and original way. This mechanism of inhibition is reinforced by production of reactive oxygen species (Matias et al., 1999b). When an antioxidant such as vitamin E is added in the cell culture medium, protein synthesis inhibition is reduced. It is likely, the specific inhibition due to EF2 phosphorylation remains. The cytotoxicity of OA has been attributed in part to induction of oxidative stress (Matias et al., 1999b; Traoré et al., 1999a,b). Oxidative stress has been shown to inhibit GJIC (Upham et al., 1997). This is in complete agreement with our data which demonstrate that OA inhibits GJIC in Caco-2 cells, similar to the effect of many other tumour promoters (Krutovskikh et al., 1995; Ren et al., 1998). This inhibition is concentration-dependent, and is correlated with parallel decreases in Cx-43 mRNA synthesis.

8-Hydroxyguanine [8-OH-dG], or 7,8-dihydro-8-oxoguanine, is a common DNA-adduct resulting from injury to DNA by reactive oxygen species. It affects the in vitro methylation of nearby cytosine moieties by the human DNA methylases, particularly methylation of cytosine residues when the 8-OH-deoxyguanosine is one or two nucleotide 3′ from the cytosine on the same strand. On the other hand, 8-OH-deoxyguanosine does not diminish the ability of the enzyme to respond to a methyl director (5-methylcytosine) when the 8-OH-deoxyguanosine is on the same strand, but one or two nucleotides from the 3' methyl director. Our data indicate that the amount of 8-OH-dG formed increased with increasing concentrations of OA, and that the rate of m5dC formation is inversely proportional. This is similar to the data of Turk et al. (1995), since when using various 8-OH-deoxyguanosine-containing oligonucleotides as substrates in methylation assays, they found that oxidative damage of parental strand guanines

would permit normal copying of methylation patterns by maintenance methylation mechanisms, while oxidative damage of guanines in the nascent strand DNA inhibited such methylation (Turk et al., 1995).

In the particular case of OA, GC→AT transversion mutations can result from the process of repairing 8-OH-dG bases induced through oxidative stress. At the same time, hypermethylation is induced at low concentrations of OA, which may lead to possible silencing of tumour suppressor gene(s). Hypomethylation is induced at higher concentrations resulting in an aberrant increase in expression of oncogenes. Thus, it appears that both mutation and epigenetic mechanisms may be involved in the initiation of the carcinogenesis process by OA. These two mechanisms are not incompatible, and indeed the proposal is in agreement with the findings and hypotheses of Baylin (1997), Counts and Goodman (1995a,b), Jones and Laird (1999). In addition GJIC is inhibited by OA, indicating that epigenetics are prominently involved in the mechanisms of carcinogenesis of this known tumour promoter. This is in agreement with the data of Klaunig (1991). It could be hypothesised that the inhibition of GJIC is due to lipid peroxidation in the cell membrane. This is indeed in agreement with the data showing that antioxidants such as vitamin E and polyphenol prevent inhibition of GJIC induced by tumour promoters (Troll and Weisner, 1985; Trosko, 1997; Klaunig et al., 2000). However, this effect would have to be specific for Cx-43, since OA induces a concomitant increase in total RNA synthesis as shown by the increase of ^{3}H-uridine incorporation in Caco-2 cells. It could also be due to DNA methylation, as shown by Boyes and Bird (1991). Thus OA known to be a tumour promoter could also be initiator. This remains to be clarified.

Acknowledgements

The study described was supported in part by the French Institute of Marine Research (IFREMER) 1 Quai du Commandant Silhouette, 33120 Arcachon (IFREMER, PNEAT, no. 96-5 411452, 1996) France and University Bordeaux 2—France. The authors are grateful to Professor Thomas SHIER, Editor of Toxin Reviews, University of Minnesota, Minneapolis (USA) for comments on the manuscript.

References

Amzil, Z., Pouchus, Y.F., Le Boterff, J., Roussakis, C., Verbist, J.F., Marcaillou-Lebaut, C., Masselin, P., 1992. Short-time cytotoxicity of mussel extracts: a new bioassay for okadaic acid detection. Toxicon 30, 1419–1425.

Baker, T.K., Kwiatkowski, A.P., Madhukar, B.V., Klaunig, J.E., 1995. Inhibition of gap junctional intercellular communication by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in rat hepatocytes. Carcinogenesis 16, 2321–2326.

Baylin, S.B., 1997. Tying it altogether: epigenetics, cell cycle, and cancer. Science 277, 1948–1949.

Berven, G., Seatre, F., Halvorsen, K., Seglen, P.O., 2001. Effects of diarrhetic shellfish toxin, okadaic acid, on cytoskeletal element, viability and functionality of rat liver and intestinal cells. Toxicon 39, 349–362.

Beyer, E.C., 1993. Gap junctions. Int. Rev. Cyt. 137, 1–37.

Boe, R., Gjertsen, B.T., Vintermyr, O.K., Houge, G., Lanotte, M., Doskeland, S.O., 1991. The protein phosphatase inhibitor okadaic acid induced morphological changes typical of apoptosis in mammalian cell. Exp. Cell Res. 195, 237–246.

Boyes, J., Bird, A., 1991. DNA methylation inhibits transcription directly via a methyl-CpG binding protein. Cell 64, 1123–1134.

Budunova, I.V., Carbajal, S., Slaga, T.J., 1996. Effect of divers tumor promoters on the expression of gap-junctional proteins connexin (Cx) 26, Cx-31.1 and Cx-43 in SENCAR mouse epidermis. Mol. Carcinog. 15, 202–214.

Cerda, S., Weitzman, S.A., 1997. Influence of oxygen radical injury on DNA methylation. Mutat. Res. 386 (2), 141–152.

Cesen-Cummings, K., Fernstrom, M.J., Malkinson, A.M., Ruch, R.J., 1998. Frequent reduction of gap junctional intercellular communication and connexin43 expression in human and mouse lung carcinoma cells. Carcinogenesis 19, 61–67.

Chang, N.T., Huang, L.E., Liu, A.Y., 1993. Okadaic acid markedly potentiates the heat-induced hsp70 promoter activity. J. Biol. Chem. 268, 1436–1439.

Ciminiello, P., Fattorusso, E., Forino, M., Magno, S., Poletti, R., Viviani, R., 1999. Isolation of 45-hydroxyyessotoxin from mussels of the Adriatic sea. Toxicon 37, 689–693.

Counts, J.L., Goodman, J.I., 1995. Alteration in DNA methylation may play a variety of roles in carcinogenesis. Cell 83, 13–15.

Counts, J.L., Goodman, J.I., 1995. Hypomethylation of DNA: a Epigenetic Mechanism that can Facilitate the Aberrant Oncogene Expression Involved in Liver Carcinogenesis. Academic Press, New York, pp. 227–255.

Fujiki, H., Suganuma, M., 1999. Unique features of the okadaic acid activity class of tumor promoters. J. Cancer Res. Clin. Oncol. 125, 150–155.

Guzman, M., Castro, J., 1991. Okadaic acid stimulates carnitine palmitoyltransferase I activity and palmitate oxidation in isolated rat hepatocytes. FEBS Lett. 291, 105–108.

Hofmanova, J., Machal, M., Kozubik, A., 2000. Epigentic mechanisms of the carcinogenic effets of xenobiotics and in vitro methods of their detection. Folia Biol. 46 (5), 165–173.

Jones, P.A., Laird, P.W., 1999. Cancer epigenetics comes of age. Nat. Genet. 21, 163–167.

Kenne, K., Fransson-Steen, R., Honkasalo, S., Warngard, L., 1994. Two inhibitors of gap junctional intercellular communication, TPA and endosulfan: different effects on phosphorylation of connexin 43 in the rat liver epithelial cell line, IAR 20. Cancerogenesis 15, 1161–1165.

Klaunig, J.E., 1991. Alteration in intercellular communication during the stage of promotion. Soc. Exp. Biol., 688–692.

Klaunig, J.E., Kamendulis, L.M., Yong, Xu, 2000. Epigenetic mechanisms of chemical carcinogenesis. Hum. Exp. Toxicol. 19, 543–555.

Krutovskikh, V.A., Mesnil, M., Mazzoleni, G., Yamasaki, H., 1995. Inhibition of rat liver gap junction intercellular communication by tumor-promoting agents in vivo. Association with aberrant localization of connexin proteins. Lab. Invest. 72, 571–577.

Kumagai, M., Yanagi, T., Murata, M., Yasumoto, Y., Kat, M., Lassus, P., Rodriguez-Vasquez, R., 1986. Okadaic acid as the causative toxin of diarrhetic shellfish poisoning in Europe. Agric. Biol. Chem. 50, 2853–2857.

Lerga, A., Richard, C., Delgrado, M.D., Canelles, M., Frade, P., Cuadrado, M.A., Leon, J., 1999. Apoptosis and mitotic arrest are two independent effects of the protein phosphatases inhibitor okadaic acid in K562 Leukemia cells. Biochem. Biophys. Res. Commun. 24, 256–264.

Matias, W.G., Bonini, M., Creppy, E.E., 1996. Inhibition of protein synthesis in a cell free system and Vero cells by okadaic acid, a diarrhetic shellfish toxin. J. Toxicol. Environ. Health 48, 101–109.

Matias, W.G., Traoré, A., Bonini, M., Creppy, E.E., 1999. Oxygen reactive radicals production in cell culture by okadaic acid and their implication in protein synthesis inhibition. Hum. Exp. Toxicol. 18, 634–639.

Matias, W.G., Traoré, A., Creppy, E.E., 1999. Variations in the distribution of okadaic acid in organs biological fluids of mice related to diarrhoeic syndrome. Hum. Exp. Toxicol. 18, 345–350.

Matias, W.G., Creppy, E.E., 1998. 5-Methyldeoxycytosine as a biological marker of DNA damage induced by okadaic acid in Vero cells. Environ. Toxic. Water Quality 13, 83–87.

Murakami, Y., Oshima, Y., Yasumoto, T., 1982. Identification of okadaic acid as a toxic component of a marine diniflagellate *Prorocentrum lima*. Bull. Jpn. Soc. Sci. Fish. 48, 69–72.

Ren, P., Mehta, P.P., Ruch, R.J., 1998. Inhibition of gap junctional intercellular communication by tumor promoters in connexin43 and connexin32-expressing liver cells: Cell specificity and role of protein kinase C. Carcinogenesis 19, 169–175.

Sueoka, E., Fujiki, H.J., 1997. Carcinogenesis of okadaic acid class tumor promoters derived from marine natural products. Cancer Res. Clin. Oncol. 123, 413–419.

Tachibana, K., Scheurer, P.J., Tsukitani, Y., Kikuchi, H., Engen, D.V., Clardy, J., Gopichand, Y., Schimitz, F.J., 1981. Okadaic acid, a cytotoxic polyether from two marine sponges of the genus *Halichondria*. J. Am. Chem. Soc. 103, 2469–2471.

Traoré, A., Ambaliou, S., Dano, S.D., Creppy, E.E., 2000. Combined effects of okadaic acid and cadmium on lipid peroxidation and DNA bases modifications (m5dC and 8-(OH)-dG) in Caco-2 cells. Arch. Toxicol. 74, 79–84.

Traoré, A., Bonini, M., Dano, D.S., Creppy, E.E., 1999. Synergistic effects of some metals containing mussels on the cytotoxicity of the marine toxin okadaic acid. Arch. Toxicol. 73, 289–295.

Traoré, A., Maeve, L., Sanni, A., Dano, D.S., Creppy, E.E., 1999. Induction of apoptosis in Human Colonic Epithelial cells (Caco-2) by Okadaic acid. Abstract of XXXVIIth European Congress of Toxicology EUROTOX 99 Oslo June 27–30. Toxicol. Lett. Suppl. 1/99, p. 73 (P202).

Troll, W., Weisner, R., 1985. The role of oxygen radicals as a possible mechanism of tumour promotion. Annu. Rev. Toxicol. 25, 509–528.

Trosko, J.E., 1997. Challenge to the simple paradigm that carcinogens are mutagen and to the in vitro and in vivo assays used to test the paradigm. Mutat. Res. 373, 245–249.

Turk, P.W., Laayoun, A., Smith, S.S., Weitzman, S.A., 1995. DNA adduct 8-hydroxyl 2′-deoxyguanosine (8-hydroxyguanine) affects function of human DNA methyltransferase. Carcinogenesis 16 (5), 1253–1255.

Upham, B.L., Kang, K.S., Cho, H.Y., Trosko, J.E., 1997. Hydrogen peroxide inhibits gap junctional intercellular communication in glutathion sufficient but not glutathion deficient cells. Carcinogenesis 18, 37–42.

Wachsman, J.T., 1997. DNA methylation and the association between genetic and epigenetic changes: relation to carcionogenesis. Mutat. Res. 14 (1), 1–8.