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SHORT COMMUNICATION

Cyclooxygenase-2, malondialdehyde and pyrimidopurinone adducts of deoxyguanosine in human colon cells

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Cyclooxygenases (COX) catalyse the oxygenation of arachidonic acid to prostaglandin (PG) endoperoxides. Activity of one of the COX isoforms, COX-2, results in production of prostaglandin E_2 (PGE_2) via the endoperoxide PGH_2. COX-2 has been implicated in the pathogenesis of colorectal cancer. Malondialdehyde (MDA) is a mutagen produced by spontaneous and enzymatic breakdown of PGH_2. MDA reacts with DNA to form adducts, predominantly the pyrimidopurinone adduct of deoxyguanosine (M_1G). Here the hypothesis was tested that COX-2 activity in human colon cells results in formation of MDA and generation of M_1G adducts. M_1G was detected in basal cultures of human non-malignant colon epithelial (HCEC) and malignant SW48, SW480, HT29 and HCA-7 colon cells, at levels from 77 to 148 adducts/10^8 nucleotides. Only HCA-7 and HT29 cells expressed COX-2 protein. Levels of M_1G correlated significantly ($r = 0.98$, $P < 0.001$) with those of intracellular MDA determined colorimetrically in the four malignant cell types, but neither parameter correlated with expression of COX-2 or PG biosynthesis. Induction of COX-2 expression by phorbol 12-myristate 13-acetate in HCEC cells increased PGE_2 production 20-fold and MDA concentration 3-fold. Selective inhibition of COX-2 activity in HCA-7 cells by NS-398 significantly inhibited PGE_2 production, but altered neither MDA nor M_1G levels. Malondialdehyde treatment of HCEC cells resulted in a doubling of M_1G levels. These results show for the first time in human colon cells that COX-2 activity is associated with formation of the endogenous mutagen, MDA. Moreover, they demonstrate the correlation between MDA concentration and M_1G adduct levels in malignant cells.

Colorectal cancer is the second commonest malignancy in developed countries and a significant cause of mortality. The epidemiological and molecular basis of colorectal cancer has been characterized in some detail and intense efforts are currently focused on applying this knowledge to improve risk assessment, screening and intervention for early stages of colorectal carcinogenesis (1). Accumulated DNA damage appears to contribute substantially to the aetiology of colorectal cancer (2). Many types of DNA damage have been observed in human tissues with several of these being related to normal cellular biochemistry, such as oxidative stress. The genotoxicity of exogenous and endogenous oxidizing agents arises either from direct damage to DNA or from reactions with other biomolecules that lead to formation of DNA-reactive electrophilic species (3). The oxidation of lipids, giving rise to products such as MDA and certain characteristic DNA adducts, provides an example of the indirect pathway. Since 'control' DNA from tissue unexposed to endogenous mutagens does not exist *in vivo*, correlative studies are necessary to relate changes in DNA adduct levels to carcinogenic events or intervention with chemopreventive agents (4).

Cyclooxygenase (COX) catalyses the conversion of arachidonic acid to PGG_2 and PGH_2 (Figure 1, step 1) and occurs as two isozymes, COX-1 and COX-2. COX-2 can be induced by tumour promoting stimuli *in vitro* (5) and by infection and inflammation *in vivo* (6). COX-2 mRNA and protein levels are markedly increased in many human colon adenomas and carcinomas in comparison to normal colon tissue (7,8). Inhibition of COX-2 causes cytostasis of colon cancer cells *in vitro* and impairs the growth of intestinal adenocarcinoma xenografts in mice (9). Therefore COX-2 inhibitors are currently under evaluation as potential colorectal cancer chemopreventive agents (1).

Malondialdehyde (MDA) can be generated during COX catalysis in human platelets and liver cells (10,11) by breakdown of PGH_2 via at least three routes (Figure 1). Consistent with this notion is the finding that high levels of MDA in colorectal cancer tissue appear to correlate with levels of prostaglandin E_2 (PGE_2) (12), a principal product of COX-2 (13). Malondialdehyde is mutagenic in bacterial and mammalian cells, and carcinogenic in rats (4). Malondialdehyde reacts with DNA under physiological conditions to form adducts, predominantly with deoxyguanosine to generate pyrimidopurinone-deoxyguanosine adducts (M_1G) (14). M_1G has also been associated with mutagenesis and has been detected in a variety of human tissues in the range of 3–150 adducts/10^8 nucleotides (4). Whilst oxidative DNA damage is known to occur in the human colon and has been linked with lipid peroxidation (15), M_1G in human colon tissue has not yet been described. M_1G levels in rat colon mucosa have recently been measured (16).

In the light of these results, we postulated that COX-2 activity may contribute to the development of the malignant phenotype in human colon cells via generation of mutagenic M_1G adducts. In order to test this hypothesis, basal levels of COX-2 and PGE_2 were compared with those of MDA and M_1G in five human colon cell lines: human non-malignant colon epithelial cells (HCEC) and adenocarcinoma SW48, SW480, HT29 and HCA-7 cell lines. Furthermore, cellular COX-2 activity was pharmacologically manipulated to study possible consequences for cellular MDA and M_1G levels.

Abbreviations: COX, cyclooxygenase; HCEC, human colon epithelial cells; MDA, malondialdehyde; M_1G, pyrimidopurinone–deoxyguanosine adduct; PG, prostaglandin; PMA, phorbol 12-myristate 13-acetate.

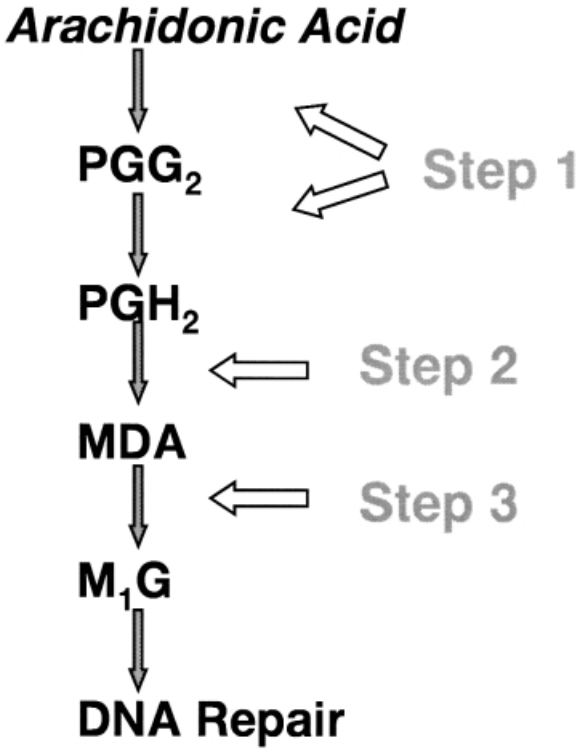

Fig. 1. Conversion of arachidonic acid to prostaglandin endoperoxides resulting in formation of MDA and potentially M_1G adducts. Step 1 incorporates both oxygenase and peroxidase activities of the COX enzyme. Step 2 represents breakdown of PGH_2 to MDA and hydroxyheptadecatrieonic acid, which can occur spontaneously or via catalysis by thromboxane synthases or other cytochromes P450 (11). Step 3 is one possible mechanism of formation of the M_1G adduct.

Non-malignant colonic epithelial HCEC cells (5) were passaged in B50 medium (Biofluids, Rockville, MD) containing bovine serum albumin, bovine pituitary extracts, retinoic acid, vitamin C and dexamethasone. Malignant colorectal carcinoma cell lines SW48, SW480, HT29 and HCA-7, which were obtained from Dr S.MacKay (University of Florida, Gainesville, FL), Prof. C.Paraskeva (Bristol University, Bristol, UK) and Dr S.Kirkland (Imperial College, London, UK), were cultured routinely in DMEM with Glutamax-1 (Life Technologies, Paisley, UK) containing 10% fetal calf serum. Experiments with all five cell types were conducted in this medium and repeated in serum-free medium to demonstrate that the presence of serum did not affect the parameters measured (data not shown). COX-2 polyclonal antibody and COX-2 protein standard were purchased from Oxford Biomedical (Oxford, UK). Anti-α-tubulin monoclonal antibody was obtained from Amersham Pharmacia Biotech (Little Chalfont, Bucks, UK). Murine M_1G monoclonal antibody D10A1, M_1G standards and NaMDA, the sodium salt of monomeric MDA, were synthesized and characterized as previously described (17,18). Anti-rabbit and anti-murine horseradish peroxidase antibodies were purchased from Dako (Ely, UK). Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma (Poole, UK) and the highly selective COX-2 inhibitor, NS-398, from Cayman (Ann Arbor, MI). Both agents were dissolved in dimethylsulphoxide before dilution in cell culture medium.

Experiments were conducted with cells grown to ~70% confluence. In order to stimulate COX-2 expression, HCEC cells were incubated with PMA (75 nM) for up to 72 h. For chemical generation of M_1G adducts, HCEC cells were treated with NaMDA (1 mM) for 24 h (19). In order to inhibit COX-2 activity, cells were incubated for up to 72 h with NS-398 at 17.7 μM, which is 10-fold higher than the reported IC_{50} value for enzyme inhibition *in vitro* (20). An aliquot (1 ml) of the culture medium was removed for measurement of PGE_2. Cells were counted after staining with trypan blue using a haemocytometer. Antioxidants that prevent lipid peroxidation were not added during workup, since previous studies have found no evidence for the generation of M_1G artifactually in hepatic and testicular tissues, with a limit of detection of 3 adducts/10^9 nucleotides (21). Malondialdehyde levels of cell lysates were assessed immediately by the colorimetric Lipid Peroxidation Assay kit (Calbiochem, San Diego, CA), which has been validated as a measure of intracellular MDA concentration in microsomes *in vitro* (22). The detection limit was ~0.1 nmol/mg protein. PGE_2 levels were determined by competitive enzyme immunoassay (Cayman); the detection limit was 10 pg/10^6 cells or 30 pg/ml culture medium. Levels were normalized with respect to cell number. COX-2 protein levels were assessed by western blotting as previously described (5). Blots were stripped and re-analysed for α-tubulin to control for protein loading and transfer. Extraction of genomic DNA and analysis of M_1G adduct levels by immunoslot blot was performed as previously described (23). The detection limit for M_1G was 5 adducts/10^8 nucleotides. M_1G results were confirmed by exchange of samples and repeat analyses at the participating laboratories in Leicester and Vanderbilt. Variation for repeat analyses of the same sample was $<10\%$. The immunoslot blot assays were validated using M_1G standards and DNA from RKO human colorectal carcinoma cells by gas chromatography–electron capture negative chemical ionization mass spectrometry, as previously described (19,23). In case PMA treatment resulted in *de novo* DNA synthesis, the M_1G data presented in Table II were normalized to the number of viable cells used for DNA extraction. Results were subjected to analyses of variance (ANOVA) and covariance with a 5% significance level using Minitab software (Minitab Inc., State College, PA). Plots of residuals were used to ensure that variances were homogeneous and that the residuals had a normal distribution.

Expression of COX protein and PGE_2 production were compared with basal levels of intracellular MDA and M_1G adducts in the five human-derived colon cell types. All the cells contained low but similar levels of COX-1 protein when corrected for protein loading (Figure 2). Detectable COX-2 protein was found only in HCA-7 cells and, to a slight extent, in HT29 cells under basal culture conditions (Figure 2). Measurable levels of M_1G adducts were detected in all five cell lines (Table I) and the differences observed between SW480, HT29, HCA-7 and SW48 cells were statistically significant ($P < 0.005$ by ANOVA). In the malignant cell types, levels of MDA correlated significantly with those of M_1G, whereas the non-malignant HCEC cells did not fit this correlation (Figure 3). There was no correlation of M_1G levels with COX-2 protein or PGE_2 production (Table I). Incubation of HCEC cells with 1 mM NaMDA for 24 h caused an increase in M_1G levels from 79 ± 25 adducts/10^8 nucleotides in control cells to 140 ± 34 adducts/10^8 nucleotides (mean ± SD; $n = 3$).

Incubation of HCEC cells with the phorbol ester PMA induced COX-2 expression (Figure 2) but did not affect COX-1 levels. It also elicited a concomitant increase in PGE_2 production, which after 4 h was ~20-fold higher than control

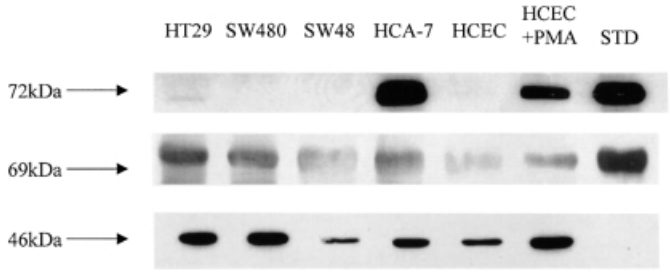

Fig. 2. COX expression in human-derived colon cell lines in basal culture and after treatment of human colon epithelial cells (HCEC) with PMA. SDS–PAGE western blots show constitutive levels of COX-2 (72 kDa) and COX-1 (69 kDa) protein in HT29, SW480, SW48 and HCA-7 colorectal carcinoma cells and non-malignant HCEC colon cells in basal culture; and in HCEC cells after incubation with 75 nM PMA for 4 h. The blots were re-probed for α-tubulin (46 kDa) to control for equal protein loading and transfer. The results shown are typical of three separate experiments. For details of western analysis see Materials and methods.

Table I. Basal cellular levels of COX-2, PGE_2, MDA and M_1G adducts

Cell type	COX-2[a]	PGE_2 (pg/10^6 cells)[b]	MDA (nmol/mg protein)[b]
HCEC	ND[c]	31 ± 20	0.38 ± 0.12
SW48	ND	122 ± 30	0.42 ± 0.18
SW480	ND	78 ± 26	0.12 ± 0.10
HT29	+[d]	410 ± 220	0.19 ± 0.10
HCA-7	+++	10 700 ± 2100	0.24 ± 0.10

[a]By Western analysis.
[b]For experimental details see Materials and methods. Values are the mean ± SD of three to five separate experiments.
[c]ND, not detectable.
[d]+, Detectable; +++, very strong band.

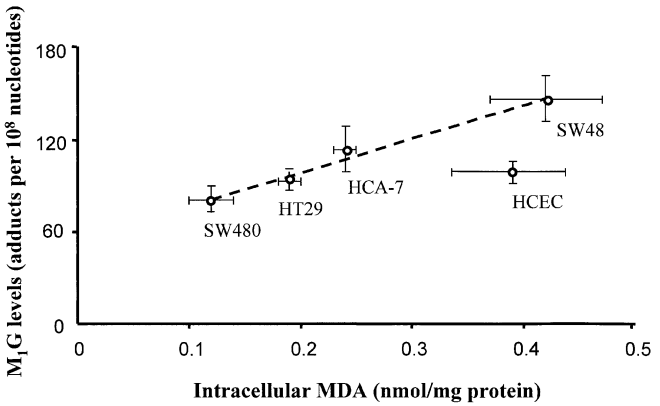

Fig. 3. Relationship between intracellular MDA and M_1G adduct levels in unstimulated culture of colon cell lines. The line of best fit ($r = 0.98$, $P < 0.001$) links values for the malignant cell types and excludes non-malignant HCEC cells. Values are the mean ± SD from three separate experiments. For experimental details see Materials and methods.

levels (Table II). COX-2 induction was also accompanied by an increase in intracellular MDA, which after 4 h was ~3-fold over control values (Table II). In contrast, M_1G adduct levels were unaffected by PMA treatment. PMA treatment of SW480 and HT29 cells also resulted in increases in COX-2, PGE_2 and MDA levels similar to those seen in HCEC cells, without significant changes in M_1G levels (data not shown). Incubation of the COX-2 expressing HCA-7 cells with the selective inhibitor NS-398 reduced PGE_2 levels from 10.7 ± 2.1 ng/10^6 cells in control cells to 1.3 ± 0.6 ng/10^6 cells after incubation for 24 h. Incubation of cells with NS-398 for up to 72 h did not alter levels of intracellular MDA or M_1G adducts.

Table II. Effect of treatment of HCEC cells with PMA on levels of cellular COX-2 protein, PGE_2 in the medium, intracellular MDA and M_1G adducts

	Incubation time (h)		
	0	4	24
COX-2[a,b]	ND[c]	+++[d]	+
PGE_2 (pg/10^6 cells)[b]	30 ± 16	745 ± 210	408 ± 170
MDA (nmol/mg protein)[b]	0.38 ± 0.12	1.07 ± 0.32	0.8 ± 0.36
M_1G (adducts per cell)[b]	4600 ± 670	5300 ± 820	4950 ± 1050

[a]Based on Western blot analysis.
[b]For experimental details see Materials and methods. Values are the mean ± SD of three separate experiments.
[c]ND, not detectable.
[d]+, Detectable; +++, very strong band.

These results describe the relationship in colon cells between MDA, M_1G adducts and COX activity, and thus contribute to our understanding of the role of COX-2 in colon cancer. We demonstrate for the first time that levels of M_1G adducts in colon cancer cells reflect constitutive intracellular levels of MDA. Similar to COX-2 protein expression, there was considerable heterogeneity in constitutive M_1G adduct levels in colorectal cancer cells, ranging from <5 adducts/10^8 nucleotides in RKO cells to 148 ± 22 adducts/10^8 nucleotides in SW48 cells. However, there appeared to be no correlation between constitutive COX-2 expression in cultured cells and levels of M_1G adducts. Similarly, inhibition and induction of COX-2 activity did not alter M_1G levels.

There are a number of possible explanations for this lack of association. The addition of exogenous MDA in high concentrations to non-malignant HCEC cells almost doubled M_1G adduct levels, to reach values similar to those observed constitutively in malignant SW48 and HCA-7 cells. The relative insensitivity of M_1G levels in cells in culture towards challenge by MDA is consistent with the possibility that routes other than intracellular production of MDA may be the major sources of M_1G formation *in vivo*, particularly since MDA is generated predominantly at the cell membrane, some distance from cellular DNA. Evidence in favour of this hypothesis was provided recently in a study of human gastric mucosal biopsies (24), in which regression analysis of samples from 39 individuals with normal gastric histology and no evidence of *Helicobacter pylori* infection demonstrated that 200% increase in MDA concentration was reflected by an average increase in M_1G of only 23%. Little is known about alternative routes of M_1G formation, but oxidation of DNA by bleomycin has been shown to give rise to M_1G adducts via base propenal formation in the absence of lipid peroxidation and MDA (25). The apparent insensitivity of M_1G adduct levels to changes in MDA concentration may also be related to rapid repair by nucleotide excision repair pathways (26).

Alternatively, the products of COX catalysis may be less efficiently converted to MDA in cultured cells than *in vivo*. We have shown previously (11) that PGH_2 can be converted to MDA by certain cytochromes P450 (Figure 1, step 2), which may be overexpressed in colon cancer tissue but poorly preserved in cells in culture. However, breakdown of PGH_2 can also occur spontaneously, resulting in modest changes in MDA concentration. In the study described here, a 20-fold

increase in PGE_2 production was associated with a 3-fold elevation in intracellular MDA. This result underlines the limitations of the *in vitro* model used here. The multiple pathways of MDA biotransformation *in vivo*, involving aldehyde dehydrogenases and reductases, cellular antioxidants and glutathione *S*-transferase isoenzymes, may also be poorly represented in cultured cells (27,28).

However, these limitations do not detract from conclusions reached regarding the other components of the pathway studied (Figure 1, steps 1 and 3). We have demonstrated that increased COX-2 activity in non-malignant and malignant human colon cells is associated with production of MDA. Moreover, basal levels of MDA correlate with those of M_1G adduct levels in malignant colon cells, and exposure of non-malignant colon cells to MDA can increase M_1G levels. In view of the convincing role of COX-2 in the pathogenesis of colorectal cancer (6) and of M_1G adduct levels as indicators of oxidative DNA damage (4), both merit investigation *in vivo* as biomarkers of colorectal carcinogenesis.

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References

1. Sharma,R.A., Manson,M.M., Gescher,A. and Steward,W.P. (2001) Colorectal cancer chemoprevention: Biochemical targets and clinical development of promising agents. *Eur. J. Cancer*, **37**, 12–22.
2. Fearon,E.R. and Jones,P.A. (1992) Progressing towards a molecular description of colorectal cancer development. *FASEB J.*, **6**, 2783–2790.
3. Marnett,L.J. (2000) Oxyradicals and DNA damage. *Carcinogenesis*, **21**, 361–370.
4. Marnett,L.J. (1999) Lipid peroxidation—DNA damage by malondialdehyde. *Mutat. Res.*, **424**, 83–95.
5. Plummer,S.M., Holloway,K.A., Manson,M.M., Munks,R.J.L., Kaptein,A., Farrow,S. and Howells,L. (1999) Inhibition of cyclo-oxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-ΦB activation via the NIK/IKK signalling complex. *Oncogene*, **18**, 6013–6020.
6. Taketo,M.M. (1998) Cyclooxygenase-2 inhibitors in tumorigenesis (Part II). *J. Natl Cancer Inst.*, **90**, 1609–1620.
7. Eberhart,C.E., Coffey,R.J., Radhika,A., Giardiello,F.M., Ferrenbach,S. and DuBois,R.N. (1994) Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology*, **107**, 1183–1188.
8. Kargman,S.L., O'Neill,G.P., Vickers,P.J., Evans,J.F., Mancini,J.A. and Jothy,S. (1995) Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. *Cancer Res.*, **55**, 2556–2559.
9. Sheng,H.M., Shao,J.Y., Kirkland,S.C., Isakson,P., Coffey,R.J., Morrow,J., Beauchamp,R.D. and DuBois,R.N. (1997) Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. *J. Clin. Invest.*, **99**, 2254–2259.
10. Diczfalusy,U., Falardeau,P. and Hammarstrom,S. (1977) Conversion of prostaglandin endoperoxides to C17-hydroxyacids by human platelet thromboxane synthase. *FEBS Lett.*, **84**, 271–274.
11. Plastaras,J.P., Guengerich,F.P., Nebert,D.W. and Marnett,L.J. (2000) Xenobiotic-metabolizing cytochromes P450 convert prostaglandin endoperoxide to hydroxyheptadecatrienoic acid and the mutagen, malondialdehyde. *J. Biol. Chem.*, **275**, 11784–11790.
12. Hendrickse,C.W., Kelly,R.W., Radley,S., Donovan,I.A., Keighley,M.R.B. and Neoptolemos,J.P. (1994) Lipid peroxidation and prostaglandins in colorectal cancer. *Br. J. Surg.*, **81**, 1219–1223.
13. Brock,T.G., McNish,R.W. and Peters-Golden,M. (1999) Arachidonic acid is preferentially metabolised by cyclooxygenase-2 to prostacyclin and prostaglandin E_2. *J. Biol. Chem.*, **274**, 11660–11666.
14. Marnett,L.J., Basu,A.K., O'Hara,S.M., Weller,P.E., Rahman,A.F.M.M. and Oliver,J.P. (1986) Reaction of malondialdehyde with guanine nucleosides: formation of adducts containing oxadrazabicyclononene residues in the base-pairing region. *J. Am. Chem. Soc.*, **108**, 1348–1350.
15. Pool-Zobel,B.L., Abrahamse,S.L., Collins,A.R., Kark,W., Gugler,R., Oberreuther,D., Siegel,E.G., Treptow-van Lishaut,S. and Rechkemmer,G. (1999) Analysis of DNA strand breaks, oxidized bases and glutathione S-transferase P1 in human colon cells from biopsies. *Cancer Epidemiol. Biomarkers Prev.*, **8**, 609–614.
16. Sharma,R.A., Ireson,C.R., Verschoyle,R.D., Hill,K.A., Williams,M.L., Leuratti,C., Manson,M.M., Marnett,L.J., Steward,W.P. and Gescher,A. (2001) Effects of dietary curcumin on glutathione S-transferase and malondialdehyde–DNA adducts in rat liver and colon mucosa: Relationship with drug levels. *Clin. Cancer Res.*, **7**, 1452–1458.
17. Sevilla,C.L., Mahle,N.H., Eliezer,N., Uzieblom,A., O'Hara,S.M., Nokubo,M., Miller,R., Rouzer,C.A. and Marnett,L.J. (1997) Development of monoclonal antibodies to the malondialdehyde–deoxyguanosine adduct, pyrimidopurinone. *Chem. Res. Toxicol.*, **10**, 172–180.
18. Chapeau,M.C. and Marnett,L.J. (1991) Enzymatic synthesis of purine deoxynucleoside adducts. *Chem. Res. Toxicol.*, **4**, 636–638.
19. Ji,C., Rouzer,C.A., Marnett,L.J. and Pietenpol,J.A. (1998) Induction of cell cycle arrest by the endogenous product of lipid peroxidation, malondialdehyde. *Carcinogenesis*, **19**, 1275–1283.
20. Barnett,J., Chow,J. and Ives,D. (1994) Purification, characterization and selective inhibition of human prostaglandin G/H synthase 1 and 2 expressed in the baculovirus system. *Biochim. Biophys. Acta*, **1209**, 130–139.
21. Chaudhary,A.K., Nokubo,M., Reddy,G.R., Yeola,S.N., Morrow,J.D., Blair,I.A. and Marnett,L.J. (1994) Detection of endogenous malondialdehyde–deoxyguanosine adducts in human liver. *Science*, **265**, 1580–1582.
22. Erdelmeier,I., Gerard-Monnier,D., Yadan,J.C. and Chaudiere,J. (1998) Reactions of N-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals. Mechanistic aspects of the colorimetric assay of lipid peroxidation. *Chem. Res. Toxicol.*, **11**, 1184–1194.
23. Leuratti,C., Singh,R., Lagneau,C., Farmer,P.B., Marnett,L.J. and Shuker,D.E.G. (1998) Determination of malondialdehyde-induced DNA damage in human tissues using an immunoslot blot assay. *Carcinogenesis*, **19**, 1919–1924.
24. Everett,S.M., Singh,R., Leuratti,C., White,K.L.M., Neville,P., Greenwood,D., Marnett,L.J., Schorah,C.J., Forman,D., Shuker,D. and Axon,A.T.R. (2001) Levels of malondialdehyde–deoxyguanosine in the gastric mucosa: Relationship with lipid peroxidation, ascorbic acid and *Helicobacter pylori*. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 369–376.
25. Dedon,P.C., Plastaras,J.P., Rouzer,C.A. and Marnett,L.J. (1998) Indirect mutagenesis by oxidative DNA damage: formation of the pyrimidopurinone adduct of deoxyguanosine by base propenal. *Proc. Natl Acad. Sci. USA*, **95**, 11113–11116.
26. Johnson,K.A., Fink,S.P. and Marnett,L.J. (1997) Repair of propano-deoxyguanosine by nucleotide excision repair in vivo and in vitro. *J. Biol. Chem.*, **272**, 11434–11438.
27. Canuto,R.A., Ferro,M., Muzio,G., Bassi,A.M., Leonarduzzi,G., Maggiora,M., Adamo,D., Poli,G. and Lindahl,R. (1994) Role of aldehyde metabolizing enzymes in mediating effects of aldehyde products of lipid peroxidation in liver cells. *Carcinogenesis*, **15**, 1359–1364.
28. Siu,G.M. and Draper,H.H. (1982) Metabolism of malonaldehyde *in vivo* and *in vitro*. *Lipids*, **17**, 349–355.

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