Micromolar Concentrations of Sodium Arsenite Induce Cyclooxygenase-2 Expression and Stimulate p42/44 Mitogen-Activated Protein Kinase Phosphorylation in Normal Human Epidermal Keratinocytes

K. J. Trouba and D. R. Germolec

Laboratory of Molecular Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Received January 7, 2004; accepted March 15, 2004

Toxicological Sciences vol. 79 no. 2 © Society of Toxicology 2004; all rights reserved.

Based on evidence that arsenic modulates proinflammatory events that are involved in skin carcinogenicity, we hypothesized that in normal human epidermal keratinocytes (NHEK) arsenic increases expression of the procarcinogenic enzyme cyclooxygenase-2 (COX-2) and that this occurs via specific mitogen and stress signaling pathways. To test this hypothesis, NHEK were exposed to sodium arsenite, and COX-2 expression, prostaglandin E2 (PGE2) secretion, mitogen-activated protein kinase (MAPK) phosphorylation, and DNA synthesis were quantified. Inhibitors of p42/44 and p38 MAPKs were used to evaluate the contribution of mitogen and stress signaling to the modulation of COX-2. Our results demonstrate that arsenite (0.005–5 μM) elevates COX-2 expression, PGE2 secretion (2.5–5 μM), and DNA synthesis (1–5 μM). Arsenite stimulated p42/44 but not p38 MAPK phosphorylation (2.5 μM), responses different than those produced by epidermal growth factor. Inhibition of mitogen-activated protein kinase kinase (MAPKK) and p38 MAPK using PD98059 (20 μM) and SB202190 (5 μM), respectively, attenuated the elevation of COX-2 protein induced by arsenite, whereas physiological concentrations of three COX-2 inhibitors (e.g., NS-398, piroxicam, and aspirin) reduced arsenite-stimulated DNA synthesis. These data indicate that arsenite elevates COX-2 in NHEK at the transcriptional and translational levels as well as increases PGE2 secretion. Compounds that inhibit COX-2 expression and activity may be useful in the scientific study, prevention, and treatment of arsenic skin carcinogenesis and deserve further investigation.

Key Words: cyclooxygenase; arsenic; PD98059; SB202190; MAP kinase.
the tumor promoter–like activity of this metalloid (Germolec et al., 1996; Vega et al., 2001).

Expression of cyclooxygenase-2 (COX-2), an inducible proinflammatory enzyme that regulates eicosanoid synthesis, often is elevated during cancer and is induced by tumor promoters, inflammatory cytokines, and growth factors (Fosslien, 2000). COX-2 is procarcinogenic as demonstrated by experiments where tumorigenesis was inhibited in COX-2 knockout mice and cancer chemoprevention studies that employed nonsteroidal anti-inflammatory drugs (NSAIDs; Vainio, 2001). Elevated eicosanoid (e.g., prostaglandin E2 [PGE2]) levels also occur in both basal and squamous cell carcinomas of the skin (Vanderveen et al., 1986) and are associated with increased metastatic and invasive tumor behavior (Cuendet and Pezzuto, 2000). Because COX-2 is proinflammatory and its overexpression sensitizes mouse skin for carcinogenesis (Muller-Decker et al., 2002), the mechanism(s) by which inorganic trivalent arsenic modulates inflammatory and proliferative events in the skin may involve aberrant COX-2 expression and activity.

Mitogen-activated protein kinase (MAPK) pathways regulate cell growth, transformation, apoptosis, and inflammation and modulate COX-2 expression and activity. The MAPK family includes c-Jun NH2-terminal kinases (JNKs), extracellular signal–regulated kinases (ERKs or p42/44 MAPK), and p38 MAPK/stress-activated protein kinases (SAPKs). MAPKs phosphorylate transcription factors (e.g., Elk-1) regulating COX-2 expression (Lasa et al., 2000) and accessory proteins (e.g., cPLA2) that play an active role in eicosanoid synthesis (Schmidlin, 2000). Arsenic stimulates MAPK signaling events (Barchowsky et al., 1999; Drobna et al., 2003; Qu et al., 2002; Simeonova et al., 2002), possibly contributing to alterations in COX-2 expression/activity (Tsai et al., 2002). However, simultaneous COX-2 modulation and MAPK activation by inorganic trivalent arsenic has not been demonstrated in epithelial cells, particularly in human keratinocytes, one of the primary targets for the induction of cancer by arsenic.

Because elevated COX-2 expression occurs in skin inflammation and cancer and keratinocytes are the primary site of COX-2 synthesis during these processes, we examined if sodium arsenite modulates COX-2 expression and PGE2 secretion in NHEK and the possible signaling mechanism(s) by which arsenite regulates COX-2 in keratinocytes.

MATERIALS AND METHODS

Reagents. Keratinocyte basal medium (KBM-2) and growth supplements were obtained from BioWhittaker/Clonetics (Walkersville, MD), and Trypsin-EDTA, Soybean Trypsin Inhibitor, and recombinant human epidermal growth factor (EGF) were obtained from In Vitrotek (Carlsbad, CA). Sodium-n-arsenite, N-(2-cyclohexyloxy-4-nitrophenyl) methanesulfonamide (NS-398), and acetysalicylic acid (aspirin) were obtained from Sigma Chemical Co. (St. Louis, MO), and P98059, SB202190, and 4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamid-1,1-dioxid (piroxicam) were obtained from Calbiochem (San Diego, CA). Arsenite stocks were prepared in deionized water, frozen at – 80°C, and used within 2 weeks. MAPK inhibitors and NS-398 were dissolved in dimethylsulfoxide (DMSO) and frozen at – 80°C prior to use. Aspirin and piroxicam were prepared freshly in ethanol and DMSO, respectively.

Tissue culture. Normal human mammary epithelial keratinocytes (NHEK), obtained from BioWhittaker/Clonetics, were cultured in monolayer at ≤ 6 in low calcium concentration (0.15 mM) growth medium (KBM-2) supplemented with EGF (5 ng/ml), bovine pituitary extract (BPE, 50 mg/ml), 0.5 mg/ml hydrocortisone, 50 μg gentamicin/ml, transferrin, and epinephrine. Cells were incubated at 37°C in a CO2-enriched atmosphere (5%). For all experiments, cells were cultured to 60–80% confluence (2 to 3 days postplating). Subsequent treatment of cells with noncytotoxic concentrations of sodium arsenite (Hamadeh et al., 2002) was performed in supplemented KBM-2 minus BPE and hydrocortisone (KBM-2 ▼) to eliminate the mitogenic effects of BPE and anti-inflammatory effects of hydrocortisone. EGF present in KBM-2 ▼ did not interfere with MAPK activation by arsenite (unpublished data). PD98059, SB202190, or vehicle (DMSO) treatment was performed at the time of medium replacement (KBM-2 ▼), 1 to 2 h prior to the addition of arsenite. In experiments employing PD98059, SB202190, aspirin, piroxicam, and NS-398, vehicle concentrations were maintained below 0.5%.

DNA synthesis. NHEK were seeded into 24-well tissue culture plates (12,000 cells/well) in KBM-2 and grown to 60–80% confluence. Medium was replaced with fresh KBM-2 ▼ containing 0, 1, 2.5, or 5 μM arsenite. Cells were labeled for 2 h with 1 μCi/ml [3H]-thymidine (Amersham Pharmacia, Piscataway, NJ) at 46 h of arsenite exposure and then washed several times with ice-cold Hank’s balanced salt solution (HBSS). Following several washes with 10% trichloroacetic acid (Mallinkrodt, Phillipsburg, NJ), radioactivity was eluted using 0.3 N NaOH and [3H]-thymidine incorporation into DNA was quantified (counts per minute, CPM, are expressed as percentages of control) by scintillation counting. In experiments employing nonsteroidal anti-inflammatory drugs (NSAIDs), medium was replaced with fresh KBM-2 ▼ containing 2.5 μM arsenite in combination with 1000 μM aspirin, 10 μM NS-398, and 10 μM piroxicam. NSAID concentrations were based on therapeutic doses as previously reported (Dromgoole et al., 1983; Futaki et al., 1993; Hobbs, 1983) and on cytotoxicity data for NHEK. NHEK cytotoxicity was quantified using the neutral red cytotoxicity assay and was not increased following coexposure to 2.5 μM arsenite plus aspirin, NS-398, or piroxicam at 1000 μM, 10 μM, and 10 μM, respectively (unpublished data).

Northern blotting. NHEK were seeded into 150-mm tissue culture dishes (300,000 cells/dish) in KBM-2 and grown to 60–80% confluence, at which time the medium was replaced with KBM-2 ▼. Following a 1 to 2 h acclimation at 37°C, arsenite was added to a final concentration of 0, 1, 2.5, or 5 μM (in duplicate). After arsenite treatment, NHEK were washed with ice-cold HBSS, cell pellets were obtained from duplicate plates, and RNA was isolated using the RNeasy mini-prep system (Qiagen, Valencia, CA). Next, 20 μg aliquots of total RNA were fractionated by formaldehyde-agarose gel electrophoresis. Following transfer to nylon membranes and UV cross-linking, membrane-bound RNA was hybridized using QuikHyb solution (Stratagene, La Jolla, CA) to a 32P-labeled human probe for human COX-2 prepared using random primers methodology (Invitrogen). Membranes were exposed to phosphoimager screens and gene expression was visualized using a Molecular Dynamics phosphoimager and Image Quant software (Amersham Pharmacia).

Western blotting. NHEK were seeded into 100-mm tissue culture dishes (100,000 cells/dish) in KBM-2 and grown to 60–80% confluence, at which time the medium was replaced with KBM-2 ▼. After a 1 to 2 h acclimation at 37°C, arsenite was added to the culture medium. Following arsenite treatment, cells were disrupted in ice-cold lysis buffer (10 mM Tris-HCl [pH 7.5], 150 mM NaCl, 5 mM EDTA [pH 8.0], 1% Triton X-100, 10% glycerol, 1 mM NaVO3, 1 mM NaF, 5 mM DTT, 10 μg/ml leupeptin, 20 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride) prior to protein quantification. Lysates were combined with NuPage LDS buffer (Invitrogen) under reducing conditions, and SDS-PAGE (15 μg cellular protein) was performed on 4–12% bis/tris gels (Invitrogen). Fractionated proteins were transferred onto Hybond (Amersham Pharmacia) nitrocellulose membranes and incubated with a goat polyclonal COX-2 antibody (Caymen Chemicals, Ann Arbor, MI). For MAPK
analysis, membranes were incubated with polyclonal antibodies for either anti-phospho/active p42/44, p38, MEK-1, -2, nonphosphorylated p38 (Cell Signaling/New England Biolabs, Inc., Beverly, MA), or nonphosphorylated p42/44 MAPK (Upstate Biotechnologies, Waltham, MA). A rabbit secondary antibody to IgG conjugated to horseradish peroxidase was used for chemiluminescence detection and proteins were visualized using the ECL detection system (Amersham Pharmacia), according to the manufacturer’s instructions. Protein expression was quantified using a Molecular Dynamics laser densitometer and Image Quant software (Amersham Pharmacia).

**PGE2 measurements.** NHEK were seeded into 12-well tissue culture dishes (15,000 cells/dish) in KBM-2 and grown to 60–80% confluence, at which time the medium was replaced with KBM-2/c141 containing 0, 1, 2.5, or 5 μM arsenite. Following treatment, culture medium was collected, centrifuged at 12,000 × g for 5 min to remove cell debris, and frozen at –80°C prior to analysis. PGE2 in culture medium was measured by enzyme immunoassay (Oxford Biomedical Research Inc., Oxford, MI), according to the manufacturer’s instructions. Quantification was performed using a Molecular Devices (Sunnyvale, CA) kinetic microplate reader and SoftMax Pro software.

**Statistical analysis.** When appropriate, analysis of variance (ANOVA) was performed following ANOVA, significant differences were determined by application of the Tukey-Kramer Multiple Comparisons Test or the Students t-test ($p \leq 0.05$). All experiments were performed a minimum of three times unless otherwise indicated.

**RESULTS**

COX-2 contributes to proliferation in keratinocytes (Rys-Sikora et al., 2000). Previous studies from our laboratory indicated that arsenite elevated *cox-2* mRNA in NHEK at concentrations relevant to those seen in arsenic-exposed pop-

FIG. 1. Sodium arsenite elevates COX-2 mRNA in NHEK. Cells were treated as described in the materials and methods. Representative northern blots showing the dose-dependent increase in *cox-2* at 4, 8, and 24 h of arsenite exposure. Twenty-eight and 18S rRNA bands from each respective ethidium bromide stained gel indicate equivalent loading of total RNA. Similar results were obtained in two or more independent experiments.

FIG. 2. Sodium arsenite elevates COX-2 protein in NHEK. Cells were treated as described in the materials and methods. (A) Representative western blots showing the dose-dependent increase in COX-2 at 8 and 12 h of arsenite exposure. (B) Densitometric quantification of COX-2 protein. Each point represents the mean of three independent experimental replicates; error bars denote SE and asterisks (*) indicate a significant difference ($p \leq 0.05$) from time-matched controls (no arsenite).
PGE₂ (0 μM arsenite) occurred over a period of 48 h, similar to that reported previously (Maloney et al., 1998). The finding that low levels of arsenite (e.g., 0.005–1 μM) failed to stimulate PGE₂ secretion, where changes in cox-2 were seen, may be due in part to COX-independent mechanisms (e.g., increased arachidonic acid release) involved in prostaglandin synthesis or post-translational modifications of COX-2 that are important in its enzymatic activity (Hoozemans et al., 2002; Shimokawa et al., 1990).

To determine if arsenite influences MAPK signaling in NHEK, p42/44 and p38 MAPK activation was examined using phosphorylation-specific MAPK antibodies. Concentrations of 2.5 (Fig. 4A) and 5 μM (unpublished data) arsenite stimulated a transient (5–30 min) and delayed (2, 3, and 4 h) increase in p42/44 MAPK phosphorylation. EGF, a growth factor that activates MAPKs in numerous cell types including keratinocytes (Medema et al., 1994), stimulated p42/44 phosphorylation in a sustained manner (Fig. 4B). Figures 4A and 4B show that the level of nonphosphorylated p42/44 following arsenite or EGF stimulation remained the same, indicating that changes in p42/44 phosphorylation were not due to alterations in steady-state protein level. Immune complex kinase assays also revealed that arsenite and EGF increased p42/44 MAP kinase activity similar to phosphorylation (unpublished data). The kinetics of MEK-1/-2 phosphorylation were similar to that of reporter gene studies.
confirming that arsenite exposure results in the activation of the kinase upstream of p42/44 MAPKs (Fig. 4A), although the mechanism is still unknown. In contrast, arsenite did not stimulate phosphorylation of the stress kinase p38 at a concentration of 2.5 μM (Fig. 5A), whereas EGF induced p38 phosphorylation within 5 min of exposure (Fig. 5B). These data indicate that arsenite stimulates rapid phosphorylation of p42/44 MAPKs, but not p38 MAPK, in NHEK.

MAPKs regulate COX-2 expression and function; therefore, the effect of arsenite on COX-2 expression in the presence of the MEK inhibitor PD98059 (20 μM) or the p38 MAPK inhibitor SB202190 (5 μM) was examined. PD98059 and SB202190 are specific inhibitors of the 42/44- and 38-kDa MAPK pathways, respectively. Both inhibitors, independently, decreased the elevation in COX-2 protein (~70 kDa) at 12 h of arsenite exposure (Fig. 6), an effect consistent with the roles of p42/44 and p38 in COX-2 regulation (Dean et al., 1999; Mifflin et al., 2002). Similar effects occurred with the MEK inhibitor U0126 and p38 inhibitor SB203580 (unpublished data). To verify that the MAPK inhibitors were specific for their intended pathways, the effects of SB202190 and PD98059 on p42/44 and p38 phosphorylation in the presence of either 2.5 or 5 μM arsenite were evaluated. PD98059 inhibited p42/44 phosphorylation, whereas SB202190 increased p38 phosphorylation (Fig. 7). SB202190 did not affect p42/44 phosphorylation, and the MEK-1 inhibitor PD98059 did not stimulate p38 phosphorylation. These data indicate that PD98059 had a specific effect on the p42/44 and SB202190 on the p38 MAPK pathways. The stimulatory effect of SB202190 on p38 phosphorylation is thought to be due to inhibition of p38 MAPK activity via a loss of negative pathway regulation (Wang et al., 2000).

Because arsenite can modulate proliferation in numerous cell types and abnormal proliferation is a key component of cancer, we studied whether a selective COX-2 inhibitor, N-(2-cyclohexyloxy-4-nitrophenyl) methanesulfonamide (NS-398), or general inhibitors of COX enzymes, 4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamid-1,1-dioxid (piroxicam) or acetylsalicylic acid (aspirin), could suppress arsenite-induced DNA synthesis. In NHEK, arsenite stimulated DNA synthesis in a dose-dependent manner after 48 (Fig. 8A) and 72 h (Germolec et al., 1996; Vega et al., 2001) of exposure. As shown in Figure 8B, DNA synthesis stimulated by 2.5 μM arsenite (at 48 h of exposure) was reduced by treatment with 1 mM aspirin, 10 μM piroxicam, or 10 μM NS-398. DNA synthesis stimulated by 5 μM arsenite also was inhibited by piroxicam and NS-398 (unpublished data). These data indicate that NSAIDs reduce the ability of arsenite to stimulate 3H-thymidine incorporation in NHEK.

**DISCUSSION**

Elevated expression and activity of the proinflammatory/-carcinogenic enzyme COX-2 occurs in numerous carcinomas including those in skin, and studies in rodent and human tissues have indicated that overexpression of COX-2 contributes to...
skin carcinogenesis (Buckman et al., 1998). Because COX-2 participates in skin carcinogenesis and is induced by a variety of cytokines, growth factors, and tumor promoters, we investigated its modulation by arsenite using an in vitro culture system.

In NHEK, sodium arsenite stimulated time- and dose-dependent changes in COX-2 expression and PGE₂ secretion. Our data are consistent with a recent report by Tsai and coworkers showing the induction of COX-2 protein, mRNA, and PGE₂ secretion in human umbilical vascular endothelial cells (HUVECs) by arsenite (Tsai et al., 2002). These authors demonstrated that modulation of COX-2 by arsenite was mediated through NF-κB. In their study and another (Bunderson et al., 2002), where arsenite-induced COX-2 expression was monitored in bovine aortic endothelial cells (BAECs), induction of COX-2 occurred at arsenite concentrations five to ten times higher than those used in our study. Our data indicate that arsenite modulates COX-2 expression in NHEK at concentrations (e.g., submicromolar to low micromolar) within the range of those found in contaminated drinking water and in the urine of humans consuming contaminated drinking water (Gebel, 2001).

Similar to previous studies, our western analyses consistently showed the presence of a protein doublet that migrated at the appropriate COX-2 molecular weight (70 kDa; Habib et al., 1993). Both bands in this doublet are thought to be COX-2; however, the higher molecular weight band is believed to represent the active form of this enzyme since COX-2 contains several tyrosine groups that are substrates for phosphorylation and are important in its enzymatic activity (Shimokawa et al., 1990). Chemical modification of specific tyrosine residues by nitration also abolishes COX-2 enzyme catalytic activity (Deeb

FIG. 6. Effect of MAPK inhibition on COX-2 modulation by sodium arsenite. NHEKs were exposed to arsenite and PD98059 (20 μM) or SB202190 (5 μM) as described in the materials and methods. Representative western blots showing COX-2 expression following treatment with DMSO (VH), PD98059 (PD), or SB202190 (SB), and arsenite for 12 h. Graphs indicate the relative change (% of control [arsenite alone at each dose]) in band density/protein expression and SE across four independent experiments. Asterisks (*) indicate a significant difference (p ≤ 0.05) from control.
reported p42/44 MAPK phosphorylation kinetics in response to keratinocyte growth factor (KGF/FGF-7) stimulation similar to those of this study; yet, the mechanism(s) and biological significance of biphasic (transient and delayed) kinase phosphorylation are not well understood. Interestingly, the kinetics of p42/44 MAPK phosphorylation stimulated by arsenite are different than those for EGF and suggest that low concentrations of arsenite initially target EGF-independent signaling in NHEK. EGF receptor–mediated signaling, nonetheless, is proposed to be a target of arsenic (Simeonova et al., 2002; Wu et al., 1999). Many studies examining the effects on MAPK signaling have employed significantly higher concentrations of arsenic (≈ 100 μM; Liu et al., 1996; Ludwig et al., 1998). Although novel data have been derived from these studies, high-dose arsenic exposure is likely to modulate stress-, apoptotic-, or death-related events—events that may be more related to acute toxicity. This is in contrast to repeated, long-term, low-dose arsenic exposure, which would be predicted to produce chronic toxicity or carcinogenesis.

Our data suggest that both p42/44 and p38 MAPK signaling contribute to the regulation of COX-2 by arsenite, as treatment with PD98059 or SB202190 prevented maximal COX-2 induction. Inhibition of MEK-1, -2, or p38 did not completely abrogate the increases in COX-2, indicating that additional events contribute to COX-2 regulation by arsenite. Chen and coworkers reported that p42/44 MAPks do not play a role in the stimulation of cox-2 expression by UVB radiation in immortalized human keratinocytes, whereas activation of p38 is required for cox-2 induction (Chen et al., 2001). These data, along with ours, indicate that the mechanisms involved in skin carcinogenesis are unique to the environmental insult and/or cell type. The finding that p38 phosphorylation is not stimulated transiently by arsenite yet treatment with SB202190 leads to a reduction in arsenite-induced COX-2 expression is difficult to resolve. One possibility is that arsenite stimulates p38 phosphorylation at a later time point(s) than those examined or, alternatively, that it induces/represses the action of a secondary mediators(s) that modulates p38 activation in a delayed manner. COX-2 induction and MAPK activation occurred at concentrations of arsenite that also stimulated DNA synthesis. NSAIDs that inhibit COX enzymes are promising chemopreventative agents for human cancers including skin cancer (Marks and Furstenberger, 2000), and it has been suggested that the inhibitory effect of some NSAIDs on carcinogenesis occurs during the tumor promotion step (i.e., during proliferation; Castano et al., 1997). Our findings indicate that aspirin, piroxicam, or NS-398 partially suppressed the stimulatory effect of arsenite on DNA synthesis and implicated COX enzymes in the modulation of keratinocyte proliferation by arsenite. Specific NSAIDs not only inhibit cyclooxygenases but also modulate mitogenic events including MAPK (e.g., ERK and JNK) and transcription factor (e.g., AP-1) activation (Huang et al., 1997; Liu et al., 2003). In some cases, MAPK
inhibition takes place when NSAID treatment precedes mitogenic stimulation (e.g., following UV irradiation) but not when treatment occurs concomitant with or following stimulation (Huang et al., 1997). In our experiments, arsenite was added concurrently with aspirin, piroxicam, or NS-398, and the stimulation of p42/44 phosphorylation by arsenite was not inhibited by pretreatment with either 10 or 30 μM NS-398 (unpublished data). The latter suggests that inhibition of early MAPK/tran-
scription factor activation is not critical in arsenite-induced DNA synthesis attenuation following NSAID treatment.

In summary, the relationship between COX-2 induction and MAPK activation presents a possible mechanism(s) involved in arsenic-induced dermatotoxicity, preneoplastic events, and skin cancer, and one that may be of particular toxicological relevance. Altered COX-2 expression, mediated partially by MAPKs, would be predicted to contribute to skin carcinogenesis by influencing inflammation, apoptosis, proliferation, differentiation, metabolism, and immunity.

ACKNOWLEDGMENTS

The authors thank Kristen Geisenhoffer, Ahmed Khan, Rachel Patterson, Christopher McPherson, and Rob Wine for their technical contributions to these studies. We are also grateful to Yvette Rebolloso, Jennifer Britton, Christina Inhof, and Drs. Wei Qu, Jennifer Ingram, Jean Harry, and Christian Lefebvre D’Hellencourt for their insightful comments concerning this report.

FIG. 8. The effect of COX-2 inhibitors on sodium arsenite-stimulated DNA synthesis in NHEK. DNA synthesis was assessed as described in the materials and methods. (A) [3H]-thymidine incorporation in NHEK following 48 h of arsenite exposure. Each point represents the mean of three biological replicates expressed as percent change; error bars denote SD and asterisks (*) indicate a significant difference (p ≤ 0.05) from the time-matched control (no arsenite). (B) NHEKs were treated for 48 hours with 2.5 μM arsenite plus different COX inhibitors (1000 μM aspirin (VH=ethanol-0.4%), 10 μM piroxicam (VH=DMSO-0.06%), or 10 μM NS-398 (VH=DMSO-0.03%)). Data are expressed as percent inhibition of each respective control (2.5 μM arsenite in the presence of vehicle) and are the mean ± S.D. of 6 biological replicates and p ≤ 0.05 (*). NSAIDS alone had a minimal inhibitory effect (< 10%) on DNA synthesis. Similar results were obtained in two independent experiments.
REFERENCES


Downloaded from https://academic.oup.com/toxsci/article-abstract/79/2/248/1649398 by guest on 16 January 2019


