A p38 MAP kinase inhibitor regulates stability of interleukin-1-induced cyclooxygenase-2 mRNA

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Received 9 October 1998

Abstract The mechanism by which p38 mitogen-activated protein kinase (MAPK) regulates the induction of cyclooxygenase (COX)-2 by interleukin-1 (IL-1) has been investigated in HeLa cells. SB 203580, an inhibitor of p38 MAPK, in the range 0.1–1 μM inhibited IL-1-stimulated PGE_2 (but not arachidonic acid) release and this was associated with inhibition of induction of COX-2 protein and mRNA. IL-1 stimulated COX-2 transcription in HeLa cells about 2-fold as judged by both reporter gene and nuclear run-on assays. The inhibitor had no significant effect on this. However, in cells previously stimulated with IL-1 it caused rapid destabilisation of COX-2 mRNA independently of on-going transcription. The results suggest a novel function for p38 MAPK in the regulation of mRNA stability.

Key words: p38 MAP kinase; Interleukin-1; Cyclooxygenase-2; mRNA stability

1. Introduction

p38 mitogen-activated protein (MAP) kinase is strongly activated by a number of stimuli including the pro-inflammatory cytokines interleukin-1 (IL-1) and tumour necrosis factor α (TNFα), bacterial lipopolysaccharide and various types of cellular stress [1,2]. It plays a role in several cellular processes, including stress-induced cytoskeletal re-organisation [3], platelet aggregation [4] and the induction of proteins associated with the inflammatory response such as cyclooxygenase (COX)-2, matrix metalloproteinases, IL-1, IL-6, TNFα, and inducible nitric oxide synthase [5–7]. These functions are reflected in part by a growing list of proposed downstream targets of p38 MAPK which include protein kinases such as MAPK-activated protein kinases 2 and 3 (MAPKAPK-2 and 3) [8] and several transcription factors such as MEF2C [9], CHOP/GADD153 [10], ATF2 [1], and ternary complex factors [11].

We have previously shown that SB 203580, a potent pyridinyl imidazole inhibitor of p38 MAPK, strongly inhibited IL-1-stimulated PGE_2 production in human fibroblasts, and that this was associated with suppression of induction of COX-2 mRNA and protein by the cytokine [6]. Similar findings have been reported for COX-2 induction in IL-1-stimulated mesangial cells [12] and LPS-stimulated monocytes [13]. We also showed that the p38 MAPK inhibitor suppressed induction by IL-1 of matrix metalloproteinases at the level of mRNA [6]. This was in contrast to the inhibitor's effect on suppression of cytokine production in LPS-stimulated monocytes which was found to be in large part translational [14,15].

We have used HeLa cells to study further the role of p38 MAPK in IL-1-stimulated prostaglandin production and COX-2 induction. Using SB 203580, we have found that p38 MAPK activity is essential for stabilising COX-2 mRNA.

2. Materials and methods

2.1. Materials

HeLa cells (human cervical epidermal carcinoma) were from ECACC, Porton Down, UK. Bacterial plasmids expressing recombinant human GST-c-Jun^{1-135} and murine His-tagged MAPKAPK-2 were gifts from Dr James Woodgett, Ontario Institute for Cancer Research, Canada, and Dr Mattias Gaestel, Max-Delbruck Centrum fuer Molekulare Medizin, Berlin, Germany. Recombinant human hsp27 was from StressGen. Anti-human COX-2 peptide antiserum was from Oxford Biomedicals. The rabbit anti-human p38 MAPK C-terminal peptide antiserum has been described [4]. Sheep anti-human MAPKAPK-2 antiserum was from Upstate Biotechnology. The *Taq*/*Pfu* mixture 'TaqPlus Precision', pBluescript II (KS^+) and a 1.5-kb human β-actin cDNA were from Stratagene. The reporter gene plasmids pCAT3 and pGL3b were from Promega. Human COX-2 cDNA (3.4 kb) was from Professor Desmond Fitzgerald, Royal College of Surgeons in Ireland, Dublin, Ireland. A cDNA (1.1 kb) for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was from Dr Chris Clark, Kennedy Institute of Rheumatology, London, UK. Primers CP1 (5′-GCGACGCTATGGTACACAATAGTCACAGTACTTTTC-3′) and CP2 (5′-GCGCAAGCTTGTCCTGACGCTCACTGC-3′) were from Genosys Biotechnologies. 4-(4-Fluorophenyl)-2-(4-methylsulfonylphenyl)-5-(4-pyridinyl) imidazole (SB 203580) was from Calbiochem. [γ-^{32}P]ATP (3000 Ci/mmol), [α-^{32}P]dCTP (3000 Ci/mmol), [α-^{32}P]UTP (800 Ci/mmol) and [^{14}C]acetyl coenzyme A (55 Ci/mmol) were from Amersham Life, [5,6,7,8,11,12,14,15(*N*)-^{3}H]arachidonic acid (185 Ci/mmol) was from NEN.

2.2. Cell culture

Adherent HeLa cells were maintained in DMEM/10% (v/v) FCS in a humidified atmosphere of 5% (v/v) CO_2 at 37°C. IL-1 stimulation and other cell treatments were also under these conditions.

2.3. Metabolic labelling of cells with [5,6,7,8,9,11,12,14,15-^{3}H(*N*)]arachidonic acid

HeLa cells were grown to approximately 70% confluence in 12-well (22 mm diameter) dishes. The medium was removed and replaced with 1 ml DMEM/10% (v/v) FCS containing 0.5 μCi [5,6,7,8,9,11,12,14,15-^{3}H](*N*)]arachidonic acid (184.6 Ci/mmol) per ml. Cells were incubated at 37°C for 16 h before being washed twice with 1 ml DMEM/FCS. Either SB 203580 or DMSO vehicle (0.01% (v/v)) was added 1 h prior to stimulation with IL-1. Medium was then removed and 10 μl sodium arachidonate (10 mM) was added per sample, before centrifugation at 6000×*g* for 1 min. Total released ^{3}H-labelled arachidonic acid and its metabolites were measured by direct liquid scintillation

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Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; IL, interleukin; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; PG, prostaglandin

0014-5793/98/$19.00
PII: S0014-5793(98)01342-8

counting of supernatants. Arachidonic acid was separated from PGE_2 by organic extraction and TLC as described [16].

2.4. Northern and Western blot analysis

These were done as described previously [6]. A phosphorimager (FLA2000, Fuji) was used for quantitation.

2.5. Construction of CP(−2300/+30)pGL3b, a reporter gene plasmid containing 2300 bp upstream of the human COX-2 promoter

High molecular-weight genomic DNA was purified from 10^8 human peripheral blood mononuclear cells which had been prepared by Ficoll gradient centrifugation. The 2.3-kb upstream region of human COX-2 promoter was amplified by 25 PCR cycles from 0.5 μg genomic DNA using 50 pmol CP1, 50 pmol CP2, 250 μM dNTPs, and 5 U *Taq*/*Pfu* mix. The PCR fragment was then subcloned into pGL3b, a commercial luciferase reporter plasmid lacking any eukaryotic or viral promoter, at unique *Mlu*I/*Hin*dIII sites generated from the respective primers. The cloned promoter region was sequenced commercially. When compared with the three regions of corresponding sequence in GenBank it matched perfectly except for two possible single-base discrepancies.

2.6. Transient transfection of HeLa cells with CP(−2300/+30)pGL3b

Cells were seeded 24 h prior to transfection into 6-well plates. 1.45 μg pBluescript (inert carrier), 0.5 μg pGL3b-CP(−2300/+30) and 0.05 μg pCAT3 (a chloramphenicol acetyltransferase reporter plasmid under the control of the SV40 promoter) were mixed with 10 μl 'Superfect' reagent (Qiagen)/well and added to the cells for 2 h, following the manufacturer's recommendations. The cells were then washed, and harvested 24–48 h post-transfection, following appropriate treatment. For harvesting, cells were lysed in 150 μl lysis buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 150 mM NaCl, and 0.65% (v/v) Nonidet (NP-40)) per well and centrifuged at 12 000×*g* for 2 min at 4°C. Luciferase and CAT were assayed by standard methods. Luciferase activities were normalised for any inter-sample transfection variation with corresponding CAT activities.

2.7. Purification of c-Jun-N-terminal kinase (JNK) by anion exchange chromatography

HeLa cells were stimulated with IL-1 (20 ng/ml, 15 min), collected by scraping and lysed by passing through 23- and 25-gauge needles. The lysate was cleared by centrifugation and chromatographed on a Mono-Q column; elution was with a linear gradient of NaCl (0–0.5 M) in 20 mM Tris-HCl buffer, pH 8.5, containing phosphatase inhibitors [17]. Two peaks of JNK activity were detected by assay on GST-c-Jun^{1-135} [17,18]. These were tested for sensitivity to inhibition by SB 203580 by pre-treating JNK for 15 min before assay.

2.8. Immunoprecipitation and assay of p38 MAPK or MAPKAPK-2

p38 MAPK was immunoprecipitated [4] and immunosorbates were assayed in kinase buffer (30 μl) containing 20 mM HEPES, pH 7.5, 20 mM Na-β-glycerophosphate, 200 mM NaCl, 2 mM DTT, 10 mM $MgCl_2$, 10 mM NaF, 0.1 mM Na-orthovanadate, 0.5 mM EDTA, 0.5 mM EGTA, 0.05% Brij35, 20 μM ATP, 0.03 mg/ml MAPKAPK-2 and 1.3 μCi/μl [γ-^{32}P]ATP. Samples were shaken for 20 min at room temperature before being boiled in SDS-PAGE sample buffer for 5 min and subjected to SDS-PAGE. ^{32}P incorporation into MAPKAPK-2 was quantified in a phosphorimager. Immunoprecipitation and assay of MAPKAPK-2 was performed in an identical manner to that for p38 MAPK, except a sheep antiserum coupled to protein G-agarose beads was used, and the immunosorbates were assayed on hsp27 substrate.

2.9. Nuclear run-on assays

These were based on the method described in [19]. 4×175-cm^2 flasks of HeLa cells were used for each treatment. Cells were scraped into phosphate-buffered saline, centrifuged (500×*g* for 5 min) and lysed by resuspension (250 μl/flask) in nuclear extraction buffer (0.14 M NaCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.6, 0.5% (v/v) NP-40, 1 mM DTT, 40 U/ml 'RNasin'). Nuclei were centrifuged (500×*g* for 5 min) washed twice and stored in liquid nitrogen as described [19]. Run-on assays and RNA extraction were performed as described [19]. Equivalent amounts of radiolabelled RNA (usually about 5×10^6 cpm/sample) were added to 2 ml hybridisation mixture (50% (v/v) formamide, 5× SSPE, 5% (v/v) Denhardt's solution, 0.1% (w/v) SDS, 0.1 mg/ml salmon sperm DNA) and hybridised to slot blots for 72 h at 42°C. Slot blots contained cDNAs of interest in pBluescript (human COX-2, human β-actin). The plasmid DNA (10 μg/slot) had been linearised, denatured, and UV-crosslinked to Hybond N as described [19]. Wash steps were also performed as described [19] and the blots were visualised by autoradiography, and quantified using a phosphorimager.

3. Results

3.1. SB 203580 inhibits PGE_2, but not arachidonate, release from IL-1-stimulated HeLa cells

HeLa cells were metabolically labelled with [5,6,7,8,9,11,12,14,15-^{3}H](*N*)-arachidonic acid and pre-treated with SB 203580 1 h prior to stimulation with IL-1 for 2 h in the continuing presence of the inhibitor. Medium was removed and its ^{3}H content determined (Fig. 1A). Increasing doses of SB 203580 inhibited the release of [^{3}H]arachidonate metabolites and separation of these by TLC revealed that they mainly comprised PGE_2. This was consistent with our earlier findings

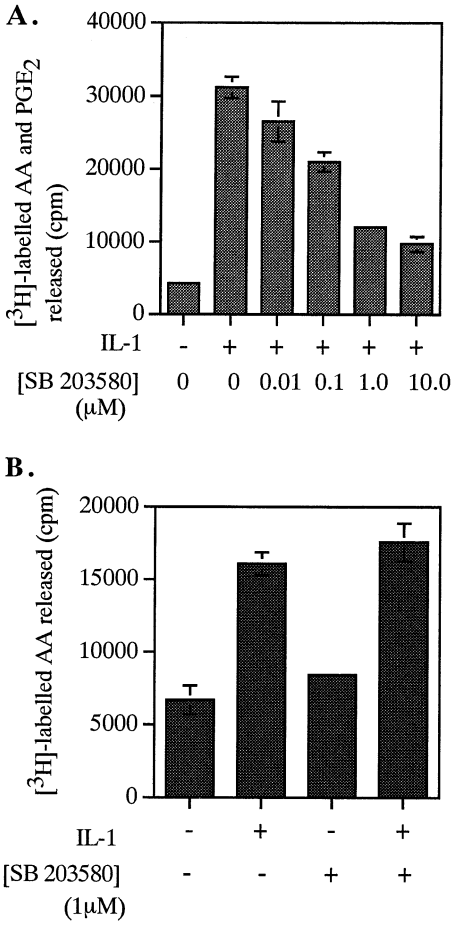

Fig. 1. SB 203580 inhibits PGE_2, but not arachidonic acid release from IL-1-stimulated HeLa cells. HeLa cells were metabolically labelled with [5,6,7,8,9,11,12,14,15-^{3}H](*N*)arachidonic acid (AA) before washing. A: Varying concentrations of SB 203580 were added 1 h prior to stimulation with IL-1 (20 ng/ml) for 2 h. Culture medium was then removed and its ^{3}H content measured directly by liquid scintillation counting. B: Cells were pre-treated for 1 h with 1 μM SB 203580 before stimulation with IL-1 for 45 min. [^{3}H]Arachidonic acid was extracted and separated from [^{3}H]PGE_2 by TLC. Representative experiments are shown. Data points are mean ± S.D. of triplicate determinations.

in human fibroblasts where IL-1-stimulated PGE_2 production (measured by radioimmunoassay) was inhibited by SB 203580 with an IC_{50} ~0.1 μM [6]. It is thought that IL-1 activates cytoplasmic phospholipase A_2 ($cPLA_2$) in cells in order to provide arachidonate as substrate for prostaglandin synthesis, and that activation may involve phosphorylation by MAP kinases. We therefore investigated the early increase in arachidonate release caused by IL-1, before significant amounts of COX-2 are induced. Medium was harvested after 45 min of stimulation of HeLa cells with IL-1. It was extracted and subjected to TLC as described in Section 2 and the radioactivity in the arachidonic acid band was measured (Fig. 1B). IL-1 approximately doubled the release of arachidonate, but this was unaffected by the p38 MAPK inhibitor. We concluded that p38 MAPK was not involved in the early mobilisation of arachidonic acid from membranes.

3.2. SB 203580 inhibits induction of COX-2 protein and mRNA

Having established that the inhibitor was acting at a level between arachidonate and PGE_2 release, we examined its effect on induction of COX-2. SB 203580 inhibited induction of COX-2 protein (Fig. 2A) and mRNA (Fig. 2B) by IL-1 with an IC_{50} in the range 0.1–0.5 μM, consistent with our observations in fibroblasts [6].

3.3. JNK activity in IL-1-stimulated HeLa cells is unaffected by 0.1 μM SB 203580

Since μM concentrations of the inhibitor have recently been shown to inhibit certain JNK isoforms [20,21] we separated IL-1-activated JNKs from HeLa cells by anion exchange chromatography as described in Section 2. Two main peaks of JNK activity were identified corresponding to the 'short' (eluting earlier) and 'long' forms [17]. The peaks were assayed in the presence of increasing doses of SB 203580: The early peak was unaffected by 1 μM, and was inhibited 15% at 5 μM (data not shown). The later peak was inhibited 10% at 1 μM and 40% at 5 μM (data not shown). Neither peak was affected by 0.1 μM inhibitor. We concluded that effects of SB 203580 observed in the 0.1–1-μM range were unlikely to be due to effects on JNKs.

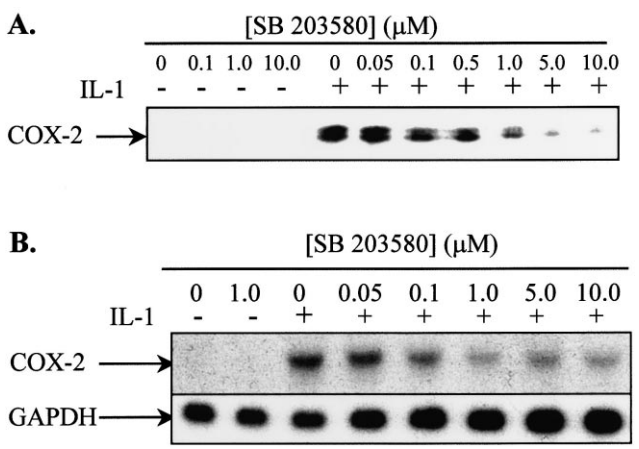

Fig. 2. SB 203580 inhibits IL-1-induced COX-2 expression in HeLa cells. HeLa cells were pre-treated for 1 h with varying concentrations of SB 203580 prior to stimulation with IL-1 (20 ng/ml). A: Western blot of COX-2 in detergent-lysed cells following 6 h stimulation with IL-1. Antigen detection was by enhanced chemiluminescence. B: Northern blots of COX-2 mRNA following 4 h stimulation with IL-1. Total cellular RNA was prepared and 5 μg/lane blotted for COX-2 and GAPDH mRNA expression with ^{32}P-labelled cDNA probes and visualised by autoradiography.

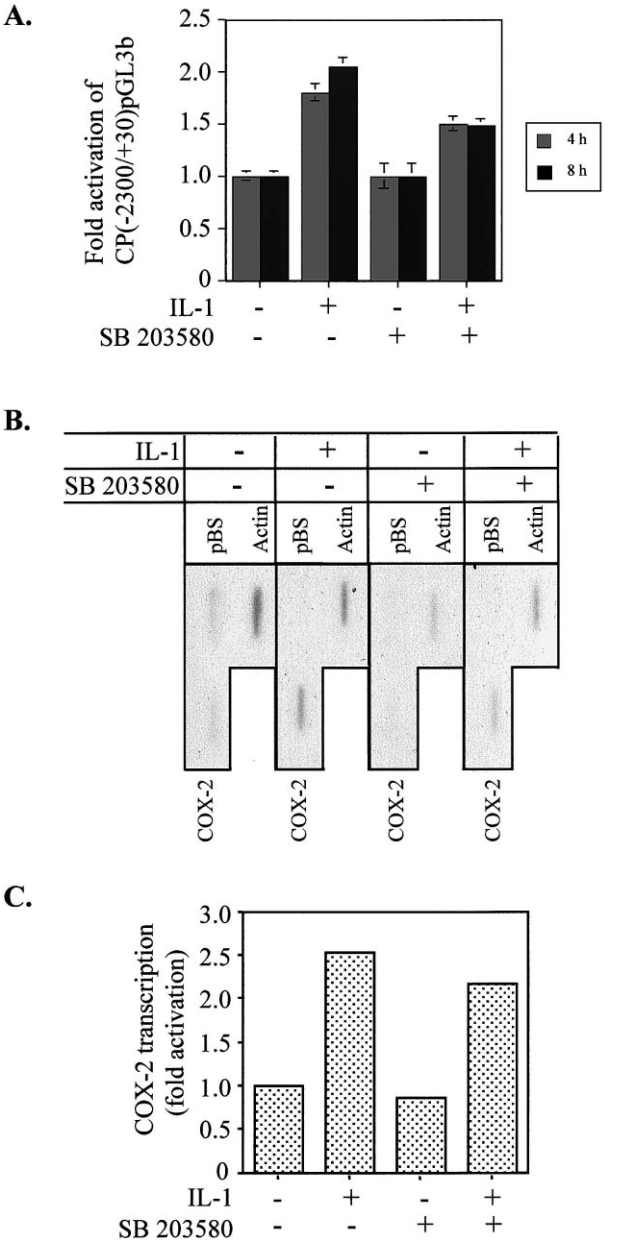

Fig. 3. The effects of SB 203580 upon IL-1-stimulated COX-2 transcription. A: HeLa cells were transiently-transfected with CP(−2300/+30)pGL3b, a luciferase reporter gene plasmid containing 2300 bp upstream of the human COX-2. Cells were pre-treated with either 1 μM SB 203580 or DMSO vehicle for 1 h prior to stimulation with IL-1 (20 ng/ml) for the times indicated. Cells were harvested (30 h post-transfection) and assayed for luciferase. A representative experiment of three is shown; data points are mean normalised luciferase activities ± S.D. of triplicate determinations. B: HeLa cells were treated for 1 h with either 1 μM SB 203580 or DMSO vehicle and were stimulated with IL-1 (20 ng/ml) for 30 min. Nuclei were then prepared and 5×10^7 from each treatment used in run-on assays in the presence of [α-^{32}P]UTP. The nascent mRNA transcripts were then extracted and hybridised to slot-blots of pBluescript (pBS) which contained cDNAs for human COX-2 or β-actin. The slot-blots were then washed and autoradiographed. Quantitation was achieved using a phosphorimager (C).

3.4. IL-1-induced COX-2 transcription is independent of p38 MAPK

The effect of the inhibitor upon COX-2 transcription induced by IL-1 was investigated in two ways.

Firstly we used a reporter gene assay. A luciferase reporter plasmid containing 2.3 kb upstream of the human COX-2 coding region was made as described in Section 2. This was transiently transfected into HeLa cells. IL-1 weakly stimulated (about 2-fold at 4 h and 8 h) the activity of the reporter gene (Fig. 3A), whilst PMA treatment caused up to 15-fold activation (data not shown). Pre-treatment with 1 μM SB 203580 resulted in partial inhibition (20%) of the IL-1-stimulated luciferase expression (Fig. 3A). Similar weak inhibition was seen in other experiments.

The results of the reporter gene assays suggested that the increase in the rate of COX-2 transcription caused by IL-1 was relatively small, and that it was only weakly regulated by p38 MAPK. We therefore investigated transcription of COX-2 more directly by nuclear run-on assay. In two separate experiments, IL-1-stimulated COX-2 transcription in HeLa cells was activated about 2-fold (relative to β-actin), and pre-treatment with 1 μM SB 203580 had no significant effect (Fig. 3B and C).

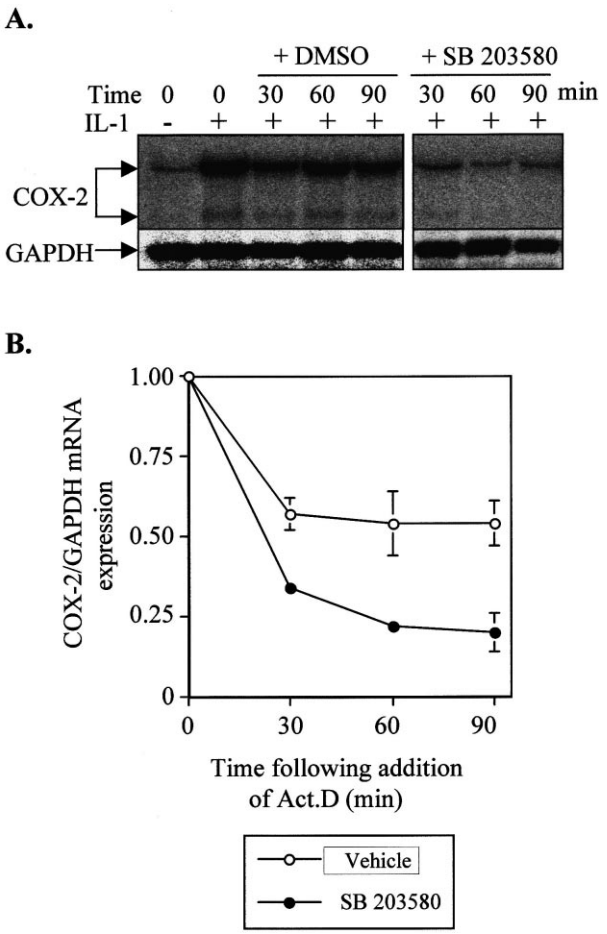

Fig. 4. SB 203580 rapidly destabilises COX-2 mRNA in HeLa cells. HeLa cells were stimulated with IL-1 for 2 h. Actinomycin D (Act.D, 1 μg/ml) with either DMSO vehicle or with SB 203580 (1 μM) was added to the cells for the times indicated. Total RNA was prepared and Northern blotted (6 μg/lane) for COX-2 and GAPDH mRNAs with ^{32}P-labelled cDNA probes which were visualised by autoradiography and quantified in a phosphorimager. A shows the autoradiograph of a representative experiment. B shows means ± S.D. derived from two independent experiments.

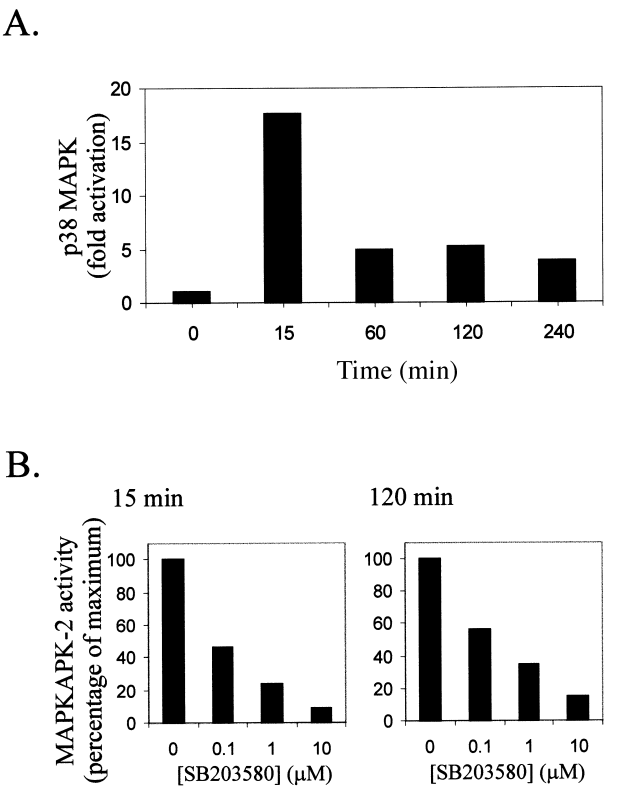

Fig. 5. Time course of p38MAPK activation (A) and inhibition of MAPKAPK-2 activation by SB 203580 (B). A: HeLa cells were stimulated with IL-1 for indicated times. Cells were washed and lysed. Lysates were treated with antiserum to p38 MAPK, and immunoprecipitates were assayed on recombinant MAPKAPK-2 as described in Section 2. ^{32}P incorporation into MAPKAPK-2 was quantified in a phosphorimager after separation of products by SDS-PAGE. A representative experiment of 3 is shown. B: Indicated doses of SB 203580 were added to HeLa cells simultaneously with IL-1 (20 μg/ml) (L H panel) or to cells which had been pretreated with IL-1 for 1.75 h (R H panel). After 15 min further incubation cells were washed, lysed and MAPKAPK-2 was immunoprecipitated and assayed on hsp 27 as described in Section 2. ^{32}P incorporation into hsp 27 was quantified in a phosphorimager after SDS-PAGE. SB 203580 (1 μM) had no effect on MAPKAPK-2 activity when added directly to the kinase assay (data not shown).

3.5. SB 203580 rapidly destabilises COX-2 mRNA in IL-1-stimulated HeLa cells

The possible effect of the p38 MAPK inhibitor upon COX-2 transcription did not account for the strong inhibition of mRNA induction. We therefore investigated the effect of the inhibitor upon the stability of COX-2 mRNA. HeLa cells were first stimulated with IL-1 for 2 h to allow accumulation of COX-2 mRNA. Following this actinomycin D was added to the cells to block any further transcription, with or without 1 μM SB 203580 (Fig. 4). In the absence of the inhibitor the mRNA decayed partially (40%) in the first 30 min, but then remained constant. However, the presence of SB 203580 strongly enhanced the decay (about 80% by 1 h). Thus in the absence of p38 MAPK activity the COX-2 mRNA became unstable.

3.6. Near to basal levels of p38 MAPK regulate COX-2 mRNA stability

In the experiment shown in Fig. 4, SB 203580 was added 2 h following IL-1 stimulation. We examined the time-course of p38 MAPK activation by immunoprecipitation kinase assay (Fig. 5A). IL-1 activation of p38 MAPK was strong at 15 min as expected. It returned towards basal level by 1 h, but remained 3-fold elevated for up to 4 h. To investigate whether or not this relatively low level of p38 MAPK at 2 h was biologically significant, we measured the effect of adding the inhibitor upon the activity of the best characterised substrate of p38 MAPK, MAPKAPK-2. Fig. 5B shows that the presence of SB 203850 at the time of maximum p38 MAPK activity (L H panel) and after return of p38 activity towards the basal level at 2 h (R H panel) inhibited p38 MAPK in the cells as judged by the reduction in MAPKAPK-2 activity at either time point. SB 203580 (1 μM) has no effect on the activity of MAPKAPK-2 itself when added directly to the kinase assay. We concluded that it is the continuing p38 MAPK activity that is required to stabilise the COX-2 mRNA, rather than the transient large increase in the initial activity caused by IL-1 stimulation of the cells.

4. Discussion

The two main conclusions to be drawn from the experiments were firstly that, as in fibroblasts, p38 MAPK activity was needed for IL-1 induction of prostaglandin synthesis in HeLa cells, and secondly, that the kinase is needed to stabilise COX-2 mRNA. Hitherto p38 MAPK has been thought to regulate transcription factors and the actin cytoskeleton and our findings suggest a new function for this kinase pathway.

We first established that stimulation of synthesis of PGE_2 and COX-2 by IL-1 was inhibited by SB 203580 in HeLa cells in a manner similar to that seen in fibroblasts [6].

We investigated whether SB 203580 had any effect on IL-1-stimulated arachidonate release. This is thought to be mediated by $cPLA_2$ which has been proposed as a substrate for p38 MAPK [22]. Our finding that the inhibitor at 1 μM had no effect on arachidonate release was consistent with our earlier observations in activated platelets [4].

The specificity of the inhibitor has recently been questioned. It was originally described as highly specific for p38 MAPK [23]. However, certain JNK isoforms (JNK2β and JNK1β) are significantly inhibited by it at 1 μM [20,21]. We therefore checked the susceptibility of HeLa JNKs to the inhibitor. The second chromatographic peak of JNK activity from IL-1-treated HeLa cells was inhibited 10% by 1 μM SB 203580. However, since significant inhibition of COX-2 expression (and PGE_2 production) was observed at 0.1 μM SB 203580, our results cannot be attributed to inhibition of JNK. Furthermore the initial evidence obtained with the inhibitor that p38 MAPK is involved in COX-2 expression has been supported by experiments in which activation of the pathway (by transfection of upstream kinases) caused COX-2 expression [24].

In order to investigate the mechanism by which the p38 MAPK inhibitor prevented COX-2 expression we first investigated transcription. There are several reports of transcriptional activation of COX-2 by IL-1. Activation ranged from 8-fold in A549 cells [25] to 15-fold in endothelial cells [26] when measured by nuclear run-on. Assays of IL-1-stimulated transcription using a reporter gene linked to the COX-2 promoter showed about a 2-fold increase in endometrial cells [27] and were unsuccessful in A549 cells [25]. Since we measured a relatively weak activation of a reporter gene in HeLa cells we also measured transcription by nuclear run-on assay. The results of this were very similar to the reporter gene assays. The 2-fold increase in transcription caused by IL-1 was not significantly affected by SB 203580 treatment in nuclear run-on assay, and the effect in the reporter gene assay was minimal.

The major effect of SB 203580 was to destabilise rapidly COX-2 mRNA. The fact that the inhibitor was effective in the presence of actinomycin D excluded any possible contribution of p38 MAPK to transcription and showed that the destabilisation occurs through modulation of extant factors. Dexamethasone has also been shown to destabilise COX-2 mRNA in IL-1-stimulated endothelial cells, but this required on-going transcription and was not seen in the presence of actinomycin D [28].

The 3′-UTR has been implicated in the regulation of COX-2 mRNA stability [29,30], but it remains to be seen whether p38 MAPK regulates COX-2 expression through this mechanism, and by which downstream targets.

In conclusion we suggest that a novel function for p38 MAPK may be to regulate mRNA stability, and it will be interesting to see if other inflammatory response genes whose expression is regulated by this kinase are controlled through a similar mechanism.

Acknowledgements: We thank the Medical Research Council of the UK, and the Arthritis and Rheumatism Council for financial support.

References

[1] Raingeaud, J., Gupta, S., Rogers, J.S., Dickens, M., Han, J., Ulevitch, R.J. and Davis, R.J. (1995) J. Biol. Chem. 270, 7420–7426.
[2] Freshney, N.W., Rawlinson, L., Guesdon, F., Jones, E., Cowley, S., Hsuan, J. and Saklatvala, J. (1994) Cell 78, 1039–1049.
[3] Guay, J., Lambert, H., Gingras-Breton, G., Lavoie, J.N., Huot, J. and Landry, J. (1997) J. Cell Sci. 110, 357–368.
[4] Saklatvala, J., Rawlinson, L., Watter, R.J., Sarsfield, S., Lee, J.C., Morton, L.F., Barnes, M.J. and Farndale, R.W. (1996) J. Biol. Chem. 271, 6586–6589.
[5] Lee, J.C. et al. (1994) Nature 372, 739–746.
[6] Ridley, S.H., Sarsfield, S.J., Lee, J.C., Bigg, H.F., Cawston, T.E., Taylor, D.J., DeWitt, D.L. and Saklatvala, J. (1997) J. Immunol. 158, 3165–3173.
[7] Da Silva, J., Pierrat, B., Mary, J. and Lesslauer, W. (1997) J. Biol. Chem. 272, 28373–28380.
[8] Cohen, P. (1997) Trends Cell Biol. 7, 353–361.
[9] Han, J., Jiang, Y., Li, Z., Kravchenko, V.V. and Ulevitch, R.J. (1997) Nature 386, 296–299.
[10] Wang, X.Z. and Ron, D. (1996) Science 272, 1347–1349.
[11] Whitmarsh, A.J., Shore, P., Sharrocks, A.D. and Davis, R.J. (1995) Science 269, 403–407.
[12] Guan, Z., Baier, L. and Morrison, A. (1997) J. Biol. Chem. 272, 8083–8089.
[13] Pouliot, M., Baillargeon, J., Lee, J., Cleland, L. and James, M. (1997) J. Immunol. 158, 4930–4937.
[14] Young, P., McDonnell, P., Dunnington, D., Hand, A., Laydon, J. and Lee, J. (1993) Agents Actions, C67–C69.
[15] Prichett, W., Hand, A., Sheilds, J. and Dunnington, D. (1995) J. Inflamm. 45, 97–105.
[16] Salmon, J. and Flower, R. (1982) Methods Enzymol. 86, 477–493.
[17] Kracht, M., Truong, O., Totty, N.F., Shiroo, M. and Saklatvala, J. (1994) J. Exp. Med. 180, 2017–2025.
[18] Kracht, M., Shiroo, M., Marshall, C.J., Hsuan, J.J. and Saklatvala, J. (1994) Biochem. J. 302, 897–905.

[19] DeWitt, D. and Meade, E. (1993) Arch. Biochem. Biophys. 306, 94–102.
[20] Whitmarsh, A.J., Yang, S.H., Su, M.S., Sharrocks, A.D. and Davis, R.J. (1997) Mol. Cell. Biol. 17, 2360–2371.
[21] Clerk, A. and Sugden, P. (1998) FEBS Lett. 426, 93–96.
[22] Borsch-Haubold, A., Kramer, R. and Watson, S. (1997) Eur. J. Biochem. 245, 751–759.
[23] Cuenda, A., Rouse, J., Doza, Y.N., Meier, R., Cohen, P., Gallagher, T.F., Young, P.R. and Lee, J.C. (1995) FEBS Lett. 364, 229–233.
[24] Guan, Z., Buckman, S., Pentland, A., Templeton, D. and Morrison, A. (1998) J. Biol. Chem. 273, 12901–12908.
[25] Newton, R., Kuitert, L., Bergmann, M., Adcock, I. and Barnes, P. (1997) Biochem. Biophys. Res. Commun. 237, 28–32.
[26] Ristimaki, A., Garfinkel, S., Wessendorf, J., Maciag, T. and Hla, T. (1994) J. Biol. Chem. 269, 11769–11775.
[27] Huang, J., Liu, D., Yadollahi, S., Wu, K. and Dawood, M. (1998) J. Clin. Endocrinol. Metab. 83, 538–541.
[28] Ristimaki, A., Narko, K. and Hla, T. (1996) Biochem. J. 318, 325–331.
[29] Gou, Q., Liu, C., Ben-Av, P. and Hla, T. (1998) Biochem. Biophys. Res. Commun. 242, 508–512.
[30] Newton, R., Seybold, J., Liu, S. and Barnes, P. (1997) Biochem. Biophys. Res. Commun. 234, 85–89.