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Interferon-α inhibits cyclooxygenase-1 and stimulates cyclooxygenase-2 expression in bladder cancer cells in vitro

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Abstract The enzymes cyclooxygenase-1 (Cox-1) and cyclooxygenase-2 (Cox-2) catalyze the initial step in the formation of prostaglandins (PGs). PGs are known to be involved in numerous processes, for example inflammation, immune responses, carcinogenesis, and tumor angiogenesis. The formation of PGs is stimulated in various cancers since the expression of Cox-2 is upregulated. Interferon (IFN)-α is used in the treatment of bladder cancer, although not all of the effects of such treatment are thoroughly known. Therefore, we investigated the expression of cyclooxygenases in two bladder cancer cell lines, 5637 and T24, under basal conditions and in the presence of human recombinant IFN-α (100, 1,000, and 10,000 U/ml). The mRNA of Cox-1 and Cox-2 was expressed in both cultured bladder carcinoma cell lines. The level of Cox-1 expression was low in 5637 cells and higher in T24 cells. In contrast, Cox-2 expression was prominent in 5637 cells and low in T24 cancer cells. The highest IFN-α concentration (10,000 U/ml) decreased the expression of Cox-1 to 47 and 28% of the control levels in 5637 and T24 cells, respectively. In contrast, Cox-2 expression increased in both cell lines. In 5,637 cells, Cox-2 expression increased 1.3-fold with 10,000 U/ml of IFN-α. In T24 cells, the maximum effect was achieved by 1,000 U/ml of IFN-α, which increased the expression of Cox-2 up to 2.4-fold. These findings may have relevance in the outcome of patients treated with IFN-α because upregulated Cox-2 expression may suppress the cell-mediated defense system. On the other hand, the inhibition of Cox-1 could be beneficial because Cox-1 is known to stimulate angiogenesis.

Key words Transitional cell carcinoma · Cyclooxygenase · Interferon-α · Eicosanoid · Arachidonic acid

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Introduction

Prostaglandins (PGs) are involved in inflammation, immune responses, ovulation, mitogenesis, and carcinogenesis [25]. The initial step in their formation is catalyzed by the cyclooxygenase enzymes, known to exist as two isoforms, namely, cyclooxygenase-1 (Cox-1) and cyclooxygenase-2 (Cox-2). Cox-1 is constitutively expressed in most tissues at a fairly stable level, whereas the low basal activity of the inducible form, Cox-2, can be increased by mitogens, cytokines, and tumor promoters [25]. Increased cyclooxygenase activity and, consequently, elevated prostaglandin levels have recently been observed in several malignancies. These malignancies include colorectal, gastric, esophageal, lung, pancreatic, hepatocellular, and breast carcinoma [8, 9, 15, 17, 22, 26, 27]. There is also strong evidence of Cox-2 overexpression in human transitional cell carcinomas of the bladder [10, 11].

Cyclooxygenases have been shown to have a role in tumor angiogenesis. Tsujii et al. [21] demonstrated that Cox-2 overexpressing colon carcinoma cells produce proangiogenic factors and stimulate both endothelial migration and tube formation in co-cultured endothelial cells. Prostaglandin E_2 (PGE_2) has also been demonstrated to suppress the cell-mediated defense system by inhibiting the binding of natural killer (NK) cells to

tumor cells [6]. This effect is also seen in bladder cancer cells [24]. In addition, non-steroidal anti-inflammatory drugs (NSAIDs) (indomethacin and ibuprofen) are reported to possess both chemopreventive and anti-invasive effects in animal studies [3, 19].

Interferons (IFNs), particularly recombinant IFN-α, have demonstrated both direct and indirect antitumoral efficacy with minimal toxicity against primary and recurrent papillary transitional cell carcinoma and carcinoma in situ. However, the response and relapse rates have been shown to be inferior to bacillus Calmette-Guérin (BCG) [1, 13, 14]. The antitumor activity of BCG and IFN-α against transitional cell carcinoma has also been demonstrated in cell cultures [13]. A recent consensus meeting suggested that recombinant IFN-α should have an important role in the treatment of superficial transitional cell carcinoma, especially following the failure of BCG or chemotherapy, and possibly in combination with other treatments [1, 12–14]. The purpose of the present study was to study the expression of Cox-1 and Cox-2 in both unstimulated bladder cancer cells and in the presence of IFN-α.

Materials and methods

Cell culture

The human urinary bladder cancer cell lines 5637 and T24 were obtained from American type culture collection (ATCC, Manassas, Va., USA). 5637 is a cell line originating from a grade 2 bladder transitional cell carcinoma and T24 originates from a grade 3 carcinoma. The cells were grown on 20.8 cm^2 petri dishes in Dulbecco's modified of Eagle's medium (DMEM), supplemented with 10% fetal calf serum (FCS). The medium was changed 24 h before initiating the experiments. When the cells reached full confluence, the medium was changed to DMEM, supplemented with 1% FCS, and the cells were divided into four different groups. The control group was treated only with fresh DMEM + 1% FCS. The other groups were treated with 100, 1,000, and 10,000 U/ml human recombinant IFN-α2A (Roceron-A, F. Hoffmann-La Roche, Basel, Switzerland), diluted in water. All groups (control and IFN-α groups) contained four identically treated cultures. The cells were then cultured at 37 °C for 24 h and harvested for the isolation of RNA.

Isolation of RNA and Northern hybridization

Total RNA was isolated from cultured bladder cancer cells as previously described [2]. The cells were lysed in 2.7 ml of 4 M guanidine thiocyanate, 25 mM sodium acetate, and β-mercaptoethanol. The lysates were layered onto a CsCl cushion (5.7 M CsCl, 25 mM sodium acetate) and centrifuged in a SW-55 rotor (Beckman, Palo Alto, Calif., USA) at 35,000 rpm (rcf 85 000*g* surface, 120 000*g* middle and 155 000*g* bottom of the tube) at 20 °C for 21 h. Thereafter, the aqueous guanidine thiocyanate solution and CsCl were removed, and the RNA pellets were washed with 99.5% ethanol. The pellets were dissolved in RNase-free water, RNA was extracted with phenol/chloroform (1:1), and the aqueous phase was precipitated with 1:10 vol 3 M sodium acetate and 99.5% ethanol at −20 °C. The RNA pellets were dried and finally dissolved in RNase-free water. Aliquots (20 μg) of total RNA were fractionated electrophoretically in 1% agarose gel after denaturation with formalin, and transferred to Zeta-probe blotting membrane (Biorad, Hercules, Calif., USA). RNA was immobilized to the membrane by baking at 80 °C for 30 min.

The RNA membranes were first hybridized with a [^{32}P]-UTP-labeled antisense cRNA probe for human Cox-2 (nucleotides 1523–1815) and later with a Cox-1 cRNA probe (nucleotides 1071–1361) [23]. The probes were generated by in vitro transcription with SP6 RNA-polymerase using the corresponding DNA fragments in pGEM-4Z vector (Promega, USA). These fragments were subcloned from the complementary DNAs, kindly provided by Dr T. Hla (American Red Cross, Rockville, Md., USA). To detect the mRNA of glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), a 1.3-kb rat cDNA probe was used [5]. For cRNA probes, the membranes were prehybridized for at least 6 h at 65 °C in a solution containing 50% formamide, 4× SSC (1× SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0), 5× Denhardt's solution, 10% dextran sulfate, 0.5% sodium dodecyl sulfate (SDS), 1 mM ethylene diamine tetra-acetic acid (EDTA), 100 mg/l heat-denatured calf thymus DNA, and 100 mg/l yeast tRNA. Hybridization was carried out at 66 °C overnight in the same solution after adding the [^{32}P]-labeled cRNA probe. After hybridization, the membranes were washed with 1× SSC and 0.1% SDS for 15 min, first at room temperature and then at 65 °C; thereafter, they were washed with 0.1× SSC and 0.1% SDS at 65 °C for 30 min. With the cDNA probe for GAPDH, the membranes were hybridized at 42 °C and washed at 60 °C. After hybridization, the membranes were exposed to phosphor imaging plates, and the intensities of Cox-1 and Cox-2 mRNA hybridization were determined using a phosphor imager plate scanner (Fujifilm, BS-5000). The data are presented as the mean ± standard error of the mean (SEM) from independent cell cultures carried out in quadruplicate. Statistical analysis was performed by an analysis of variance (ANOVA) using Fisher's protected least significant differences test.

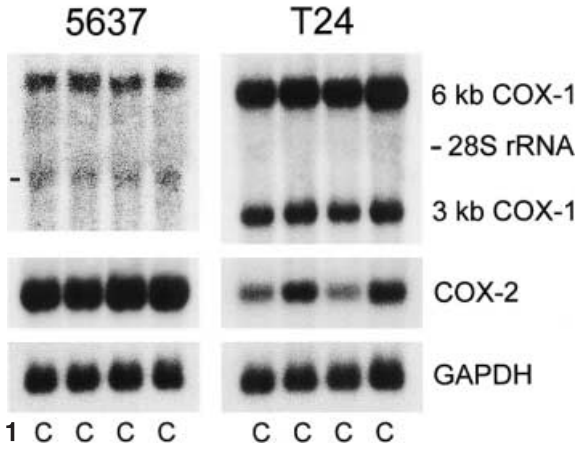
Results

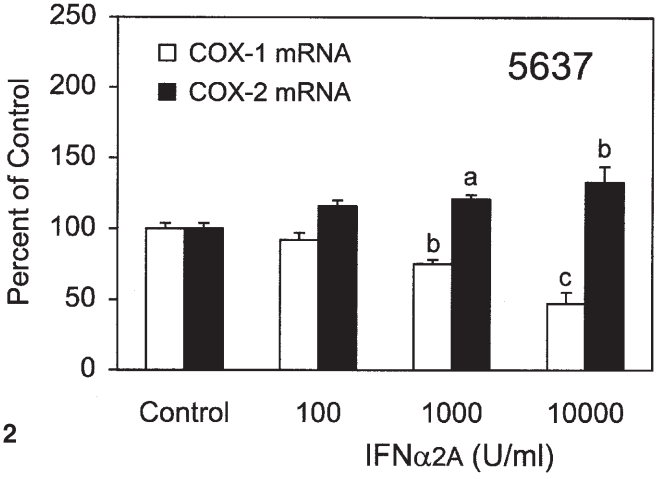
Different basal levels of cyclooxygenase expression in 5637 and T24 bladder carcinoma cell lines

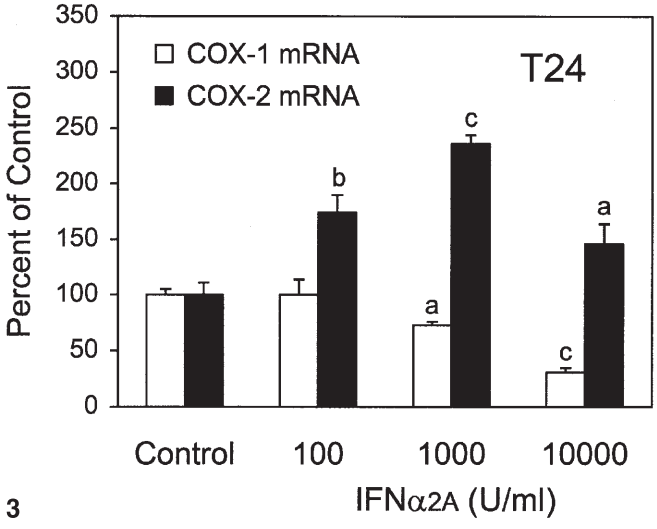
In cultured 5637 bladder carcinoma cells the basal expression of Cox-2 mRNA was prominent, whereas that of Cox-1 mRNA was weak. In addition, only the longer 6-kb transcript of Cox-1 was detected (Fig. 1). In contrast, in T24 bladder carcinoma cells, the basal expression of Cox-1 was high and two mRNA transcripts (~3 and ~6 kb) were detected (Fig. 1). The basal expression of Cox-2 mRNA was clearly detectable in T24 cells, although it was less prominent than that in 5637 cells (Fig. 1). The size of the Cox-2 mRNA transcript was about 5 kb in both 5637 and T24 cells.

IFN-α inhibits Cox-1 and stimulates Cox-2 expression in bladder carcinoma cells

In 5637 cells IFN-α treatment (100, 1,000 or 10,000 U/ml for 24 h) decreased the expression of Cox-1 in a dose-dependent manner to 75 ± 3% (mean ± SEM) and 47 ± 8% of the control level by 1,000 and 10,000 U/ml of IFN-α, respectively (Fig. 2). Although the basal expression of Cox-2 was high in 5637 cells (Fig. 1), IFN-α treatment was able to increase the level of Cox-2 mRNA 1.2–1.3-fold over the basal level (Fig. 2). Similarly, in T24 bladder carcinoma cells IFN-α treatment decreased the expression of Cox-1 in a dose-dependent manner. The combined amount of the two Cox-1 transcripts decreased to 73 ± 3% and 28 ± 6% of the control level







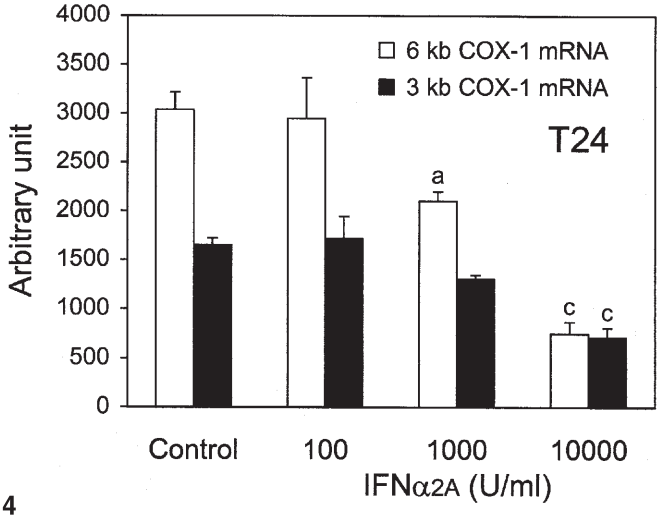


Fig. 1 Northern hybridization of the mRNAs of Cox-1 and Cox-2 in cultured 5637 and T24 bladder cancer cells. The expression of GAPDH is shown as evidence for equal loading of the RNA. Results from four control (*C*) experiments are shown. The migration of the 28S rRNA is indicated with a *line*

Fig. 2 The effect of IFN-α on the expression of Cox-1 and Cox-2 mRNA in 5637 bladder cancer cells. Results are expressed as a percentage of the corresponding control level. The mean ± SEM values from quadruplicate experiments are presented. **a** = $P < 0.05$; **b** = $P < 0.01$; and **c** = $P < 0.001$

Fig. 3 The effect of IFN-α on the expression of Cox-1 and Cox-2 mRNA in T24 bladder cancer cells. The amount of Cox-1 mRNA indicates the combined amount of 3- and 6-kb mRNA transcripts of Cox-1. Results are expressed as a percentage of the corresponding control level. The mean ± SEM values from quadruplicate experiments are presented. **a** = $P < 0.05$; **b** = $P < 0.01$; and **c** = $P < 0.001$

Fig. 4 The effect of IFN-α on the expression of 3- and 6-kb transcripts of Cox-1 in T24 bladder cancer cells. Results are expressed in arbitrary units compared to corresponding controls: **a** = $P < 0.05$; **b** = $P < 0.01$; and **c** = $P < 0.001$

by 1,000 and 10,000 U/ml IFN-α, respectively (Fig. 3). Downregulation was seen in the levels of both Cox-1 transcripts by IFN-α, although the decrease was clearer in that of the longer transcript (Fig. 4). The expression of Cox-2 was increased to 1.7- and 2.4-fold of the control level by 100 and 1,000 U/ml IFN-α, respectively (Fig. 3). With the highest concentration of IFN-α (10,000 U/ml), the expression of Cox-2 tended to level off.

Discussion

The present study indicates that Cox-1 and Cox-2 are expressed in cultured 5637 and T24 bladder carcinoma cell lines. The predominantly expressed cyclooxygenase seems, however, to be different in 5637 and T24 cells. The expression of Cox-2 was dominant in 5637 cells originating from a grade 2 bladder carcinoma. However, in T24 cells originating from a grade 3 bladder carcinoma, Cox-1 was the predominantly expressed

cyclooxygenase. Cox-2 is expressed in various carcinomas and is reported to modulate the production of angiogenic factors by colon cancer cells [21]. On the other hand, in vascular endothelial cells Cox-1 is shown to regulate and enhance the angiogenic potential of endothelial cells [21]. Consequently, both Cox-1 and Cox-2 may have a role in the formation of new blood vessels, which is essential for tumor growth. The fact that Cox-1 and/or Cox-2 were very clearly expressed in both examined bladder carcinoma cell lines needs further study.

IFN-α decreased the expression of Cox-1 in both 5637 and T24 bladder carcinoma cells. On the other hand, IFN-α stimulated the expression of Cox-2 in both cell lines. IFN-α was previously reported to possess an antiproliferative effect on T24 cells, whereas no significant effect was detected in 5637 cells [7, 14]. The inhibition of Cox-1 expression in T24 cells may be related to the antiproliferative effect of IFN-α since Cox-1 is the predominantly expressed cyclooxygenase in T24 cells. The expression of Cox-2 was not significantly enhanced in T24 cells by the highest dose of IFN-α (10,000 U/ml), although lower doses of 100 and 1,000 U/ml caused a significant increase. This may be due to the decreased vitality of T24 cells in the presence of a high concentration of IFN-α.

Dimethylprostaglandin E_2 ($dmPGE_2$), a stable derivative of PGE_2, has been shown to increase the expression of Cox-2 and the formation of PGE_2 in cultured human prostatic carcinoma cells and to stimulate cellular proliferation [20]. Thus, PGE_2 may act as a stimulatory autocrine growth factor if Cox-2 is expressed. The inhibition of Cox-2 enzyme decreases the growth of human colon cancer cells [18]. Therefore, the expression of Cox-2 in cancer is often considered to be a deleterious factor [16].

Both cyclooxygenase inhibitors and IFNs have previously been shown to have inhibitory effects on tumor growth [3, 4, 19]. Droller and Gomolka [4] used indomethacin and poly I:C (an IFN inducer) treatment in chemically induced urinary bladder carcinoma in Fisher rats. Although both agents had inhibitory effects on tumor growth, the effect was more pronounced in the poly I:C group. However, the combination of indomethacin and poly I:C had the greatest inhibitory effect.

The increased expression of Cox-2 with IFN-α treatment in both investigated bladder carcinoma cell lines is interesting and needs further study, especially in vivo. The altered expression pattern of cyclooxygenases might explain, at least in part, the failure of IFN-α treatment in some bladder cancer patients. In addition, the inhibition of Cox-1 may also be important because Cox-1 has been shown to enhance the angiogenic potential of endothelial cells.

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