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Role of Cyclooxygenase-2 Enzyme in Human Transitional Cell Carcinoma of the Urinary Bladder

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Abstract: Cyclooxygenase (COX) is considered a key enzyme in the synthesis of prostaglandins from arachidonic acid. The enzyme is believed to be involved in the inflammation, proliferation and differentiation of cells. The generated form of COX, COX-2, is increased in colonic carcinoma. To evaluate the importance of COX-2 in tumor development in the urinary bladder, the expression of COX-2 in transitional cell carcinoma of the bladder was examined to determine the usage of selective COX-2 inhibitor treatment targeting COX-2. Sixty patients with pathologically diagnosed invasive transitional cell carcinoma of the urinary bladder (pT2-pT4) were evaluated. Immunohistochemical staining was used to evaluate COX-2 expression and cases with staining of ≥10% of tumor cells were defined as positive. The results indicated that in 5 patients, 0 % of the primary tumors stained for COX-2, while 1-5 % was stained in 20 patients, 6-10% in 7 patients and ≥10% in 28 patients (28/60, 46.7%). In terms of grade, 4 patients with grade 2 (4/6, 66.6%) and 24 patients with grade 3 (24/54, 46.7%) were COX-2 positive. When categorized by stage, 16 patients with PT2 (16/33, 48.5%), 9 patients with pT3 (9/20, 45%) and 3 patients with pT4 (3/7, 42.9%) were positive. Lymph node metastasis was observed in 15 patients; 4 of them, with pN2, were COX-2-positive.Those with COX-2-positive metastatic lymph nodes had grade 3 primary tumors, which were also COX-2- positive. In addition, COX-2-negative metastatic lymph node patients also had negative primary tumors. The results of this study suggested that 46.7% of patients with invasive bladder cancer may show benefit from treatment with selective COX-2- inhibitors targeting COX-2 and that treatment efficiency can be expected in patients with lymph node metastasis when their primary tumors are COX-2-positive.

Key words: COX-2 enzyme · Histopathological grade · Invasive transitional cell carcinoma of urinary bladder · Primary tumor

INTRODUCTION

COX helps in catalyzing the conversion of arachidonic acid to prostaglandins by two different COX isoenzyme forms, COX-1 and COX-2 (1). COX-1 is heavily expressed in most tissues and mediates the synthesis of prostaglandins needed for normal physiological functions. COX-2 is not detectable in most normal tissues, but it is induced by cytokines, growth factors, oncogenes and tumor promoters. The enzyme is believed to be involved in the inflammation, proliferation and differentiation of cells [2-6]. Most epidemiological and animal studies have suggested that non-steroidal anti-inflammatory drugs, COX inhibitors, reduce the risk of colorectal cancer [7, 8, 9]. Recent studies demonstrated that the inactivation of COX-2 and treatment with a COX-2 inhibitor in APC mutant mice, a model of human familial adenomatous polyposis, significantly reduce the incidence of intestinal polyps [10]. This provided the first attention that COX-2 can play a key role in tumorigenesis. Recently, COX-2 has been found to be overexpressed in tumors in the colon, stomach, lungs and pancreas, suggesting an important role for COX-2 in tumorigenesis [11, 12, 13]. Selective COX-2 inhibitors, which only inhibit COX-2, have been developed. Their anti-proliferative effect has taken a great deal of attention [14].

Therefore, the aim of this study is to evaluate COX-2 expression by immunohistochemical (IHC) staining to investigate the possible effectiveness of selective COX-2 inhibitor treatment in patients with invasive bladder cancer.

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MATERIALS AND METHODS

Sixty patients were evaluated with pathologically diagnosed invasive transitional cell carcinoma of the urinary bladder (pT2-pT4), who were examined at Pathology Department, Cairo University (Kasr Al-Aini hospital). Fifty patients underwent radical cystectomy and 10 TUR-Bt for diagnosis. Patient age ranged from 45 to 74 years (median 61.5±7.4 years). Fifty-five were male and 5 were female. In terms of pathological grade, 6 patients were grade 2 and 54 were grade 3. Regarding T classification, 33 patients were pT2, 20 were pT3 and 7 were pT4. Regarding N classification, 34 were pN0, 6 were pN1, 9 were pN2 and 11 were pNx. The World Health Organization classification system (15) was used for the evaluation of histopathological grade and TNM classification was used for the evaluation of the primary tumor and lymph node metastasis. This study was carried out on archival specimens provided as paraffin-embedded tumor tissue obtained from the Pathology Department, Cairo University (Kasr Al-Aini Hospital School). Patients consent was not taken due to difficulty to access them after discharge from hospital. Table 1 shows the characteristics of the 60 patients.

Table 1: Patient characteristics

No. of patients		60
Age		
Range		45-74
Average		61.5±7.4
Median		61
Gender	Male	55
	Female	5
T classification	pT2	33
	pT3	20
	pT4	7
N classification	pN0	34
	pN1	6
	pN2	9
	pNx	11
Grade	G2	6
	G3	54

Immunohistochemistry Method and Evaluation: COX-2 was detected by immunostaining using labeled streptavidin biotin method. Paraffin blocks of the specimens fixed with 20% formaldehyde were prepared from 4 µm sections. Slides were deparaffinized using xylene and hydrated with graded ethanol. Endogenous peroxidase was inactivated with 3% hydrogen peroxide in absolute methanol for 30 min at room temperature. Antigen retrieval was performed 4 times for 5 min each time using a microwave in a 1-mol/l concentration of EDTA (pH 8.0) followed by washing in deionized water. Staining was performed using an automated staining apparatus for IHC (Ventana NX System, Ventana Medical System, Inc., Tucson, AZ, USA) according to the manufacturer's quidelines. Non-specific reactions were suppressed with the Endogenous Biotin Blocking Kit (Ventana Medical System, Inc.). Sections were treated with rabbit anti-human polyclonal COX-2 antibody (IBL Co., Ltd., Takasaki City, Gunma, Japan) diluted 1:25 in Tris-bovine serum albumin overnight at 4°C. The sections were subsequently washed with phosphate-buffered saline (PBS). Biotin-labeled mouse anti-rabbit IgG antibody was allowed to react at 37°C for 30 min, after which the specimens were washed in PBS, then allowed to react with horseradish peroxidase- labeled streptavidin at 37°C for 30 min. After washing, color was developed using 0.5% diaminobenzidine and 0.01% hydrogen peroxide. The sections were counterstained with hematoxylin and mounted on slides. For negative controls, the primary antibody was omitted from the samples. Inflammatory lymphoid tissue was used as a positive control. Staining of the cytoplasm of <10% of the tumor cells was considered a COX-2-negative result, while staining of ≥10% cells was defined as COX-2 positive. Each tissue specimen was examined on two separate occasions by the pathologist blinded to the stage and grade of the tumor (Fig. 1and 2).

Statistical Analysis: For statistical analysis, Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) software version 10.0 for Windows was used. The x^2 test was used to detect statistically significant differences between groups, with a level of significance of $P<0.05$.

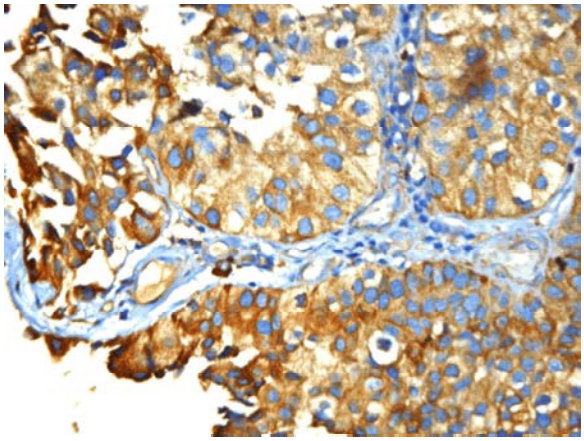

Fig. 1: Strong staining of cytoplasm in >70% of grade 3 pT3 tumor cells (COX-2-positive immunostaining x 200).

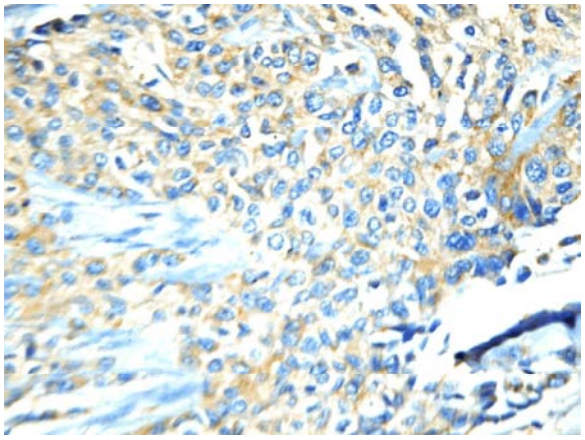

Fig. 2: COX-2 immunohistochemical staining in <5% of grade 3 pT3 tumor cells (COX-2-negative immunostaining x 200).

RESULTS

The primary tumors of 5 patients had COX-2 staining in 0% of the cells,20 in 1-5%,7 in 6-10% and 28 in ≥10% (28/60,46.7%). As shown in Fig.1 and 2, COX-2 expression was seen as light brown staining in the cytoplasm of tumor cells, whereas no staining was observed in the nuclei. In terms of grade, 4 patients with grade 2 (4/6, 66.6%) and 24 with grade 3 (24/54, 44.4%) were COX-2 positive. According to stage, 16 patients with pT2 (16/33, 48.5%), 9 with pT3 (9/20, 45%) and 3 with pT4 (3/7, 42.9%) were COX-2 positive. Fifteen patients had lymph node metastasis. Of these, 3 had COX-2 staining of 0%, 8 of 1-5% and 4 of ≥10% (4/15, 26.7%). No patients with pN1 were COX-2 positive and only 4 with pN2 were COX-2 positive (4/9, 44.4%). No statistically significant difference was observed between any of the groups (p=0.94, 0.91, 0.54) (Table 2). However, COX-2 positive patients with lymph node metastasis also had positive primary tumors and COX-2 negative patients with lymph node metastasis also had negative primary tumors. A statistically significant difference was observed between primary COX-2 positive patients and metastasis COX-2-positive patients (p=0.002).This finding is significant since it suggests that metastatic lymph node metastasis are likely to be COX-2 positive when the primary tumor is COX-2 positive too (Table 3).

Table 2: Expression of COX-2 by grade and stage

Item	COX-2 positive No. (%)	COX-2 negative No. (%)	Total	P-value
Grade				
G2	4 (66.6)	2 (33.3)	6	--
G3	24 (44.4)	30 (55.6)	54	0.94
T classification				
T2	16 (48.5)	17 (51.5)	33	--
T3	9 (45.0)	11 (55.0)	20	--
T4	3 (42.9)	4 (57.1)	7	0.91
N classification				
N1	0 (0.0)	6 (100.0)	6	--
N2	4 (44.4)	5 (55.6)	9	0.54

Table 3: Expression of COX-2 in primary tumors and lymph node metastases.

Primary tumors	Positive	Negative
Lymph node metastases (n=15)		
Positive	4	0
Negative	0	11

P= 0.02

DISCUSSION

Nowadays, the standard treatment for invasive bladder carcinoma without metastasis is in most cases radical cystectomy. However, the outcome of treatment with radical cystectomy depends widely on the pathological stage and extent of lymph node metastasis by the time of surgery. The survival rate is reported to be as high as 70%, when invasion is limited to the muscle layer (pT2). However, when peripheral fatty tissue is invaded (pT3), this decreases to 30-40% and it drops to 20% when lymph node metastasis is observed [16]. In addition, it is reported that most recurrences after radical cystectomy are of distant metastasis, with local metastasis comprising 10% [17]. Therefore, in order to accentuate the results of treatment in radical cystectomy, it is more important to eradicate micro-metastasis that cannot be diagnosed by imaging than to improve the cure rate and that's why additional treatment is required.

The approved treatment for bladder carcinoma that is unresectable or metastatic is combination chemotherapy. This treatment reported a response rate of 72% and a complete remission rate of 36% [18, 19]. Long-term survival cannot be seen due to the short duration of the response. Since most patients are elderly and require dose reduction, this regimen can cause problems in terms of dose intensity and its high toxicity may show a great hazard to patients with bladder carcinoma that are mostly elderly people [20, 21, 22]. However, the aim of treatment of cancer involves the administration of drugs targeting cancer-specific changes. Conventional chemotherapeutic agents mainly affect the nucleic acid synthesis process, DNA and microtubules and demonstrate an anti-tumor effect. These drugs lack tumor selectivity since they affect

both normal and tumor cells. Therefore, the highest tolerated dose is considered to be the optimal dose when administering conventional chemotherapeutic agents. On the contrary, targeted treatment agents generally have lower toxicity than conventional chemotherapeutic agents and are thus better able to treat elderly patients safely. In addition, they can be co-administered with conventional chemotherapeutic agents [23].

The mechanism of elevated COX-2 expression in tumor cells may depend on the activation of oncogenes [24, 25, 26]. Activation of the K-ras oncongene is associated with an elevated expression of COX-2 and the K-ras oncongene is frequently activated in bladder tumors [27]. This particular mechanism may help explain the level of COX-2 expression found in bladder tumors. COX activates many carcinogens, one of which binds directly to hot spots for mutation in the p53 gene in lung [28] and bladder cancer. Thus, COX may be involved in tumorigenesis by inactivating tumor suppression genes such as p53. COX-2-selective inhibitors may provide an alternative approach for the treatment of invasive bladder cancer. COX-2-selective inhibitors suppress colon cancer growth in vitro by inducing apoptosis, dependent and independent [29, 30] of COX-2 inhibition and suppress tumorigenesis in experimental models including rat bladder tumors induced by N-butyl-N-(4-hydroxybutyl)nitrosamine [31]. It's worth noting that, the targeted treatment of cancer requires identification of the target and is expected to be effective in only limited cases. In this study, we evaluated COX-2 expression to explore the possible effectiveness of treatment with selective COX-2 inhibitors targeting COX-2 for invasive bladder cancer. The significance of COX-2 has mainly been studied in colon cancer, with one study showing the survival rate of patients with decreased COX-2 expression to be significantly higher than that of those with increased expression. Animal studies using mice and rats have revealed that the administration of high-dose selective or non-selective COX-2 inhibitors reduces the incidence of bladder cancer and another study has reported a reduced risk of bladder cancer in NSAID users [31, 32].

In this study, overall positive staining for COX-2 was found in 46.7% (28/60) of invasive bladder cancer patients and in 26.6% (4/15) of patients with lymph node metastasis. The frequency of COX-2 expression did not show a significant correlation with grade, pathological stage or lymph node metastasis. In addition, immunostaining was limited to the cytoplasm of bladder carcinoma cells. Thus, the effect of COX-2 on invasive bladder cancer may be shown by selecting a lesion that is markedly influenced by COX-2. When considering selective COX-2 inhibitor treatment, an antitumor effect cannot be expected if 10% or more of the carcinoma is not affected. Our results were so close to that obtained by Yamada et al. [33] who reported positive staining for COX-2 in 47.5% (19/40) of invasive bladder cancer patients and in 20% (2/10) of patients with lymph node metastasis. Komhoff et al. [34] also reported that the expression of COX-2 increased with the grade and stage of bladder cancer. Yoshimura et al. [35] evaluated COX-2 mRNA expression in normal bladder, bladder carcinoma and chronic cystitis using reverse transcription polymerase chain reaction. The results demonstrate a positive correlation between the frequency of COX-2 expression and the grade and stage of disease. Shirahama [36] reported the results of COX-2 immunostaining in 35 patients with transitional cell carcinoma of the bladder and observed COX-2 expression in 20% of pT1 carcinomas and 45% of carcinomas with muscle layer invasion by immunblotting, suggesting that invasive cancer have increased COX-2 expression. He additionally reported that 93% of carcinoma in situ (CIS) showed COX-2 expression. Moreover, Mohammed *et al.* [37] reported that 86% of invasive transitional cell carcinomas, 78% of non-invasive transitional cell carcinomas and 75% of CIS were COX-2 positive. In addition, in 53% of cases, morphologically normal epithelium adjacent to the cancer lesion was COX-2 positive. They reported that this indicates that morphologically normal epithelial cells can acquire mutation and biological alteration like cancer cells and may change into tumor cells due to a paracrine effect caused by increased cytokines and/or growth factors. It was suggested that this phenomena occurred as an expression of the 'field effect' and that COX-2 expression may play a role in the pathogenesis of carcinoma. However, in our study COX-2 expression was not seen in normal cells surrounding the cancer lesions and further examination is needed. On the contrary, another study evaluating COX enzyme activity, in transitional cell carcinoma patients, supported the opposing view with an increase in enzyme activity of 70% of cells in transitional cell carcinoma cell line being observed. In addition, low-grade and low-stage carcinomas exhibited high COX enzyme activity compared to high-grade and high-stage carcinomas. Ristimaki *et al.* [13] detected COX-2 immunoreactivity in 66% of tumor cells in transitional cells carcinomas of the urinary bladder, compared to 25% in non-neoplastic samples. COX-2 immunoreactivity was localized in neoplastic cells. They reported that there was

no significant difference in the rate of positivity between invasive and non-invasive carcinomas. Shariat *et al.* [38] also measured COX-2 immunoreactivity in the cytoplasm of bladder carcinomas and reported no association between COX-2 expression and clinical findings, pathological grade, stage of lymphatic involvement. This report is in accordance with our results. In this study, it was not possible to us to analyze the relationship between COX-2 and prognosis due to inavailability of patients. According to Shirahama *et al.* [39], COX-2 expression is not a prognostic factor. However, Kim *et al.* [40] reported that COX-2 expression could predict recurrence and progression of T1, grade 3 bladder carcinoma. More evaluation with larger number of cases can be more beneficial.

CONCLUSION

Our study suggest that 46.7% of invasive bladder carcinoma patients may benefit from treatment with selective COX-2 inhibitors and that these drugs may be effective in patients with lymph node metastases when the primary tumor shows COX-2 expression. Further study leading to the establishment of effective treatment with selective COX-2 inhibitors for invasive bladder carcinoma is highly suggested. It appears worthwhile to investigate whether intravesical instillation therapy using COX-2 inhibitors is safe and effective for the treatment of bladder carcinomas because intravesical but not oral administration of the agents will allow the usage of high concentrations of the agents, which will be sufficient to kill cancer cells.

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