Is there a place for practical chemoprevention of colorectal cancer in light of COX-2 heterogeneity?

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COX-2 overexpression is widely recognized as an accidental and relatively important factor in the progress of colon neoplasia, but the practical significance of it has not yet been defined. As such, the purposes of the study were: an analysis of the changes of COX-2 expression within colon adenomas in the dependence of progress of dysplastic level within colon adenomas, the analysis of COX-2 expression in cryptal and superficial parts of polyp and, additionally, the analysis of the COX-2 heterogeneity between colon adenomas.

One hundred and four cases with completely resected adenomas with high-grade epithelial dysplasia were included in the research. Each polyp had persistent low-grade dysplasia and normal colon mucosa at the base as an internal control. Immunohistochemical analysis with monoclonal COX-2 antibody was performed.

Regression of COX-2 expression in high-grade colon intraepithelial lesions (HGCoIN) compared with low-grade colon intraepithelial lesions (LGCoIN) (p = 0.00001) was observed. No correlation between stromal COX-2 expression and either LGCoIN or HGCoIN was found (p > 0.05). The next important observation was a difference in superficial and cryptal COX-2 expression (p < 0.001) and the evident heterogeneity of COX-2 expression among adenomas at LGCoIN as well HGCoIN foci (p < 0.01).

The regression of COX-2 expression in high-grade parts of adenomas which we described may result in a reduction of the role of chemoprevention by the use of NSAIDs.

Key words: COX-2, colon neoplasia, colon adenoma, NSAID chemoprevention.

Introduction

Colorectal cancer (CRC) is one of the most frequent malignancies, currently nearly at the same level in Western and Eastern countries. Many cohort studies show that the CRC morbidity rate has a close association with socio-economic status, especially life style and diet [1-4]. There, are a few geographic differences of CRC frequency, for example, an increased percentage of right-sided tumor especially among female patients as well as younger patients in Eastern countries [2]. Many investigations have shown the possibility of different courses of early stages of carcinogenesis and potential cross-relationships between
these ways of carcinogenesis with dietary factors and acquired local inflammatory disturbance. In a few recent cohort studies the role of dietary factors such as red meat, coffee, alcohol, lack of vitamins, fiber, fruits, vegetables and the Mediterranean style diet has remained under discussion, and it is noteworthy that partially contradictory results have been published [2, 5-10]. Mechanisms which activate the “first and second hits” are still unclear, and it seems that besides the APC gene, β-catenin gene mutations or disturbances in the Wnt/β-catenin signaling pathway there exist other starting signals. In sporadic CRC, these activation signals are often connected with dietary factors and the inflammatory pathway or hypermethylation. The central Wnt/β-catenin pathway has a destruction complex which regulates the stability of cytoplasmic β-catenin and plays a dominant role in the signaling output of the canonical Wnt cascade. The tumor suppressor protein axin acts as the base of the destruction complex, interacting with β-catenin, the tumor suppressor proteins APC and WTX, and two active serine-threonine kinases (CK1α,β and GSK3α,β). In addition to its role in the Wnt pathway, β-catenin performs a second, very important, unrelated role in many epithelia. It is an essential binding partner for the cytoplasmic tail of various cadherins, such as E-cadherin in adhesion junctions. Recently, a study showed the existence of a “new Wnt/β-catenin pathway” where relocation of axin can result in β-catenin accumulation and translocation to the nucleolus [11, 12]. Hypermethylation due to dietary factors or by N-acetyltransferase 1 and 2 (NAT1, NAT2) genetic polymorphism can result in the destruction of complex abnormality with β-catenin overexpression [13, 14]. Another, interesting pathway to CRC, with evident dietary connections, involves changes of gap junctional intercellular communication (GJIC) such as connexin 43 (gap junction α-1 protein) and its influence on the inflammatory response by TGFβ-1 [15].

Arachidonic acid metabolism through cyclooxygenase, lipooxygenase, cytochrome P-450 and epoxygenase leads to overproduction of eicosanoids and plays an important role in the progress of colon neoplasia [16]. Longstanding clinical experience shows that the risk of CRC is statistically lower among people chronically using non-steroidal anti-inflammatory drugs (NSAIDs) [17, 18]. This chemopreventive effect has a source mainly in a reduction of cytoplasmic prostaglandin production and its accumulation. The NSAID action increases phosphorylation β-catenin, thus decreasing its nuclear accumulation and transcription of Wnt/β-catenin target genes such as the cyclin D1 gene and c-myc [19].

The purpose of the present study is to analyze the changes of COX-2 expression depending on dysplastic level, cryptal/superficial part of polyp and the heterogeneity of its activity.

**Material and methods**

One hundred and four cases (72 male [69%], 32 female [31%]) with completely resected colon adenomas with high-grade dysplasia were included in the research. Each polyp had persistent low-grade dysplasia and normal colon mucosa at the base as an internal control. Colon evaluations by colonoscopy were performed within the scope of the Polish National Screening Program among symptom-free patients. It was important that patients did not report use of any kind of NSAIDs chronically and did not have a familial history of CRC.

Totally resected adenomas were fixed in 10% buffered formalin and were then processed with routine histopathological procedures. Samples embedded in paraffin blocks were cut at 5 μm, mounted on slides and stained routinely with hematoxylin-eosin.

The immunohistochemical analysis using mouse monoclonal COX-2 (Novocastra, NCL-COX-2) antibody was performed. Typical immunohistochemical procedures were performed with 5 μm thickness tissue samples cut off from the FFPE blocks. After deparaffinization and rehydration of the samples, the unmasking processes, incubation with primary antibody diluted 1 : 100, and subsequent routine steps were performed using the Ventana ultra View Universal DAB Detection Kit (Ventana Medical Systems; Roche Group, Tucson, USA).

As recommended by the manufacturer, the colon biopsies with active ulcerative colitis were used as a positive control for testing the primary body.

This study using human tissues was in concordance with the ethical standards of the Declaration of Helsinki with its latest revision in 2004. Additionally, the study was approved by the Ethical Commission of the Faculty of Health Science of the Jan Kochanowski University in Kielce. All participants were informed about the nature of this anonymous study before their inclusion in the study.

COX-2 expression was obtained in part with high-grade colon intraepithelial lesions/high-grade dysplasia (HGoIN), low-grade colon intraepithelial lesions/low-grade dysplasia (LGoIN) and normal colon epithelium, and it was graded on a point scale. This part of the study has served as an analysis of the changes of COX-2 within progress of dysplasia and its heterogeneity between all the polyps. Together, but additionally, we have analysed COX-2 expression in superficial and cryptal parts of polyps.

We have assumed that for the chemopreventive effect of NSAIDs, moderate or strong expressions and wide distribution of COX-2 as potential targets for the NSAIDs’ action are important. To eliminate a false overexpression cytoplasmic amount of COX-2 in normal colon epithelium as well in inflammatory cells, we decided to use a 1 : 100 dilution of antibodies.
To reflect the quantitative COX-2 expression on sections of the adenoma’s area we used a 4-point scale:

- 0 points – moderate to strong expression in 0-25% of the adenoma’s surface,
- 1 point – moderate to strong expression in 25-50%,
- 2 points – moderate to strong expression in 50-75%,
- 3 points – moderate to strong expression above 75%.

Comparative analyses by STATISTICA ver. 10. software based on Pearson’s $\chi^2$ test, Wilcoxon’s rank test and Mann-Whitney’s $U$ test were focused on searching for dependences as well as expectations of the COX-2 expression in colon adenomas.

**Results**

The average age of patients was 66 years, and the mean diameter of polyps was 17 mm. Polyps were located as follows: right colon 25, left colon 65, rectum 14. Tubular adenomas comprised 65 cases, tubulo-villous adenomas 37 and serrated adenomas 2 cases (Table I).

We performed a comparative analysis of COX-2 in different areas of the polyp with categorization as LGCoIN, HGCoIN foci, superficial and cryptal location, respectively. Here, we observed the prominent regression of COX-2 activity in HGCoIN compared with LGCoIN (Wilcox’s test p = 0.00000). A confirmation of this results was conducted by $\chi^2$ analysis of expected COX-2 activity (Pearson’s test p = 0.05). A strictly fading of COX-2 expression with progress of dysplasia level is shown in Figures 1C-D. Figures 2 and 3 illustrate at axis X a differentiation COX-2 expression in the dependence of localisation in adenoma parts. Moreover, a quantitative comparison of the number COX-2 positive cases, including the strong examined COX-2 expression (axis Y), is demonstrated here. The median of COX-2 expression declines from level 2 in LGCoIN to level 1 in HGCoIN.

A lack of correlation of stromal COX-2 expression in stromal inflammatory cells with both LGCoIN (p = 0.09) and HGCoIN (p = 0.11) was demonstrated. The differences in superficial and cryptal COX-2 expression were another important observation. Here, we noted a substantial superficial COX-2 dominance compared with cryptal activity. This statistically significant observation is concerned only with superficial regions with low-grade dysplasia (p < 0.0001).

A consideration in this analysis of high-grade dysplasia in superficial adenoma regions with a previously demonstrated decline of COX-2 expression makes the statistical analysis insignificant (p > 0.05) (Fig. 1B). To conclude, the differences between COX-2 expression in cryptal and superficial parts of the adenomas

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**Fig. 1.** A) Superficial dominance of COX-2 expression (original magnification 40×). B) Strong expression of COX-2 at cryptal LGCoIN and gradual weakness of expression in high-grade dysplasia foci in superficial part of polyp (original magnification 40×). C, D) Gradual loss of COX-2 expression at the superficial HGCoIN lesions (original magnification 100×)
have been shown here, but only at low grade dysplasia level (Fig. 1A).

The multivariate linear regression model of analysis did not reveal correlations between routine clinical data such as age, sex, polyp size, colon localization, histopathological type and COX-2 expression ($p > 0.05$).

The next key purpose of our study was to estimate COX-2 heterogeneity among colon adenomas. We demonstrated important diversity of COX-2 expression among adenomas at LGCoIN as well HGCoIN foci ($p < 0.01$). Of note, 10% of LGCoIN adenoma parts did not show evident COX-2 expression (0 points on the used scale) in comparison with 39% of HGCoIN parts (Table II). Similar results were observed in the dependence of cryptal/superficial localization of COX-2. As previously mentioned, we observed weak cryptal activities but prominent superficial activities of COX-2 (Table II).

We observed significant heterogeneity between analyzed adenomas in the range of LGCoIN foci ($p < 0.1$), HGCoIN foci ($p < 0.1$) and expression in a superficial location ($p < 0.1$).

### Discussion

In recent years, many studies have been focused on epidemiological data, experimental models, co-

| Table I. General clinical and pathological data compared with COX-2 expression |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| N (TOTAL 104) | LGCoIN | HGCoIN | Stromal expression | Cryptal activity | Superficial activity |
| Localization |  |  |  |  |  |
| right | 25 |  |  |  |  |
| left | 65 |  |  |  |  |
| rectum | 14 |  |  |  |  |
| Sex |  |  |  |  |  |
| male | 72 |  |  |  |  |
| female | 32 |  |  |  |  |
| Type |  |  |  |  |  |
| tubular | 65 |  |  |  |  |
| villous-tubular | 37 |  |  |  |  |
| serrated | 2 |  |  |  |  |
| Age (years) | mean 66 (10.3 SD) |  |  |  |  |
| Size of polyp | mean 17 mm (12 SD) |  |  |  |  |

| COX-2 in LGCoIN |  |  |  |  |  |
| Wilcox |  |  |  |  |  |
| $p = 0.09$ |  |  |  |  |  |
| Pearson $\chi^2$ |  |  |  |  |  |
| $p = 0.05$ |  |  |  |  |  |

| COX-2 in HGCoIN |  |  |  |  |  |
| Wilcox |  |  |  |  |  |
| $p = 0.00001$ |  |  |  |  |  |
| Pearson $\chi^2$ |  |  |  |  |  |
| $p = 0.05$ |  |  |  |  |  |

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<th>Table II. Heterogeneity of COX-2 expression according to dysplastic level and superficial/cryptal parts of polyps</th>
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<tr>
<td>Heterogeneity</td>
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<tr>
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hort observations, molecular and mathematical models and clinical observations of COX-2 but, to date, guidelines for using NSAIDs do not exist in daily practice.

COX-2 overexpression in many cancers has been a widely accepted observation about arachidonic acid metabolism and its influence on many targets such as inflammatory processes, angiogenesis, migration and extracellular remodeling with metastatic potential. The pathway with the central role of COX-2 and production of prostaglandin E2 (PGE2) and next prostaglandins D2 and H2 is well recognized, and it is accepted that a target of its action is the \( \text{Wnb}/\beta\text{-catenin} \) signaling pathway \([19-21]\). It is clear that selective, as well as unselective, COX-2 inhibitors play an important role in inhibition of early stages of cancer progress. The experimental work of Zhang et al. has focused attention on alternative ways of inhibition of cellular concentration of PGE2 by 11\(\beta\)-hydroxysteroid dehydrogenase type II and the role of glucocorticoids too, as a chemopreventive factor \([22]\). The key role of arachnoid acid metabolites does not finish with PGE2. Namely, Qualtrought et al. have described the role of prostaglandin F (2\(\alpha\)) as a PGE2-derived factor which is able to promote tumor growth by an autocrine effect \([23]\). Yuri et al. and Zuo and Shureigi presented a slightly different point of view. These authors showed that COX-2 and 15-lipoxygenase-1 (15-LOX-1) have opposing impacts on colon neoplasia. They indicated that COX-2 promotes, while 15-LOX-1 inhibits, colon neoplasia. These disturbances of balance between COX-2/15-
LOX-1 with downregulation of 15-LOX-1 have been examined partially in patients with familial adenomatous polyposis syndrome (FAP) [24, 25]. The interesting mathematical and modeling work of Li and Mansmann has shown the holistic, multifactorial nature of early colon carcinogenesis and its precise connection with inflammatory process metabolites. Focused on the NSAID and miRNA-regulation pathway, the authors have shown associations with many inflammatory cytokines, with gene activation and expression of many proteins, metabolites or complexes and intersecting pathways which are able to promote or modify the neoplasia process [21].

We were looking for publications focused on examination of the differences in COX-2 expression in colon adenomas. Some authors have described the differences between adenomas and other non-adenomatous colon polyps and have reported higher COX-2 activity in polyps with HGeIN [26-28]. These papers have not discriminated between superficial and cryptal activity and changes of COX-2 activity according to the dysplastic level and these changes into each adenoma. Additionally, we have observed significant differences in the methodology of used COX-2 dilution (1:25 to 1:100) and comparative analysis between no neoplastic and neoplastic polyps. Therefore, the results from their observations differ from ours. However, the results of Marszalek et al. concerning the significance of COX-2 in stromal inflammatory cells are similar to ours. Decreasing COX-2 activity in colon cancer has been observed by Kenney et al. among young CRC patients [29]. This reported phenomenon of decreasing and fad COX-2 activity is similar to our results. The many different pathways of colon neoplasia partially explain the heterogeneity of COX-2 expression. Barry et al. described COX-2 gene polymorphism and related changes of COX-2 expressions with aspirin use [30]. The authors concluded that the mechanism of aspirin action is still unclear and there are different possible COX-2 independent pathways, such as COX-1 or LOX.

Besides COX-2, other inflammatory signaling pathways exist. They also lead to early stages of colon neoplasia and are able to be modified by NSAIDs. Here are indicated iNOS, Jak3, Stat3 and NF-κB (p65) [31]. Asting et al. focused on defining external cell signaling and transcription factors relating to high COX-2 expression in colon cancer cells. High COX-2 expression has been connected with large differences in gene expression compared with normal colon mucosa and low COX-2 expression cells [32]. This observation indicates an association of the extracellular environment with the impact on activation of the intracellular pathway and is in concordance with the theory of dietary factors. It seems that our observation of prominent superficial dominance of COX-2 expression is an indirect link to it. Furthermore, in correlation with dietary factors, cuisine styles, cultural conditioning, life style or obesity, COX-2 heterogeneity can partially explain the different results of cohort studies concerning dietary influence worldwide. Despite the lack of a consensus in the use of NSAIDs, the same clinical experiences have significance in the treatment and prognosis. Namely, Rahman et al. have shown high COX-2 expression as a marker of poor clinical outcome. In vitro as well as clinical examinations have shown higher chemosensitivity for 5-fluorouracil in combination with celecoxib [33]. It was noted that high COX-2 expression in cancer tissue is associated with a 68% increased risk of mortality.

To sum up, this paper highlights the complexity of early colon carcinogenesis in the COX-2 pathway. Besides the large input of the research in explaining the role of arachnoid acid metabolites in neoplasia, to date its contribution in daily practice is not clearly defined. An awareness of the many, though often incomplete, understandings of pathways in early colon carcinogenesis raises doubts over the blind use of NSAIDs.

The action of NSAIDs and many years of observation of clinical outcomes in patients treated with a low dose of aspirin have shown indirectly the efficiency of this form of prevention. Therefore, we suggest evaluating COX-2 expression routinely in cases of colon adenoma or carcinoma, especially in cases of non-radical polypectomy or in “partially lost polyps” cases. Owing to the fact that the number of CRCs is still growing and changes in diet/life style are unnoticeable, we propose chemoprevention by NSAIDs if COX-2 overexpression is noted. The significance of chemoprevention in patients without COX-2 overexpression is unclear, and we believe that these cases should be analyzed individually with testing of the potential advantages and complications of NSAIDs’ chronic preventive treatment. At this point in time, we see a need for a wider discussion about the practical guidance of COX-2 inhibitors.

The limitations of this study should be noted. Our results are based on an ethnically and diet-style homogeneous group.

Conclusions

The study makes an important observation about the changes of COX-2 expression within colon adenomas. Here, the decreasing of COX-2 expression with progress of epithelial dysplasia were obtained. The regression of COX-2 expression in high-grade parts of adenomas and prominent heterogeneity of COX-2 in adenomas may result in a reduction of NSAIDs’ chemopreventive role. Since the contribution of COX-2 is well documented in early colon carcinogenesis, hence it is possible that the role of COX-2 as a part of the Wnt/β-catenin pathway, from high
grade epithelial dysplasia to invasive cancer, in some cases is clearly confined.

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References


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