

RESEARCH COMMUNICATION

Cyclooxygenase-2 Expression in Gastrointestinal Stromal Tumours

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Abstract

Background: While it is well known that cyclooxygenase-2 (COX-2) expression is increased in colorectal adenoma and carcinoma, there is only limited information on its status in stromal tumours. **Methods:** Immunohistochemical COX-2 staining was performed on a total of 42 confirmed gastrointestinal stromal tumours (GISTs) in the Pathology Department of Gaziantep University and the findings were compared with various other parameters. **Results:** We found a statistically significant correlation between the tumor mitosis and COX-2 expression in GISTs. However, there was no relationship between COX-2 expression and death rate, presence of metastasis, tumour size, risk staging, usage of tyrosine kinase inhibitors, and complete resection rate. **Conclusions:** In the light of these findings, the usage of COX-2 inhibitors with or without tyrosine kinase inhibitors in GIST patients may be helpful in the adjuvant setting to prevent or delay recurrence. Moreover, we need more studies to define the status of COX-2 inhibitors in GISTs.

Keywords: GIST - COX-2 - immunohistochemical staining - prognosis - mitosis rate

Asian Pacific J Cancer Prev, 11, 849-853

Introduction

Gastrointestinal stromal tumors (GIST) are included in a group of cancers called soft tissue sarcomas. Sarcomas are among the rare tumors that originate from the cells constituting the connective tissue (muscle, fat, nerve, vessel, bone and cartilage). GIST may occur in any part of the digestive tract from the esophagus to the anus. They originate from the connective tissue that supports the digestive organs, called stroma (Miettinen et al., 2001). There are two subtypes of the cyclooxygenase (COX) enzyme: COX-1 and COX-2. They both catalyse the oxidation of arachidonic acid, thereby converting it to prostaglandins, prostacyclin and thromboxane. They are inhibited by aspirin and non-steroid anti-inflammatory drugs (Sheehan et al., 2003). The increase in COX-2 expression is known to be tumorigenic (Asano et al., 2002). In addition, COX-2 expression was increased in gastrointestinal cancers (Sano et al., 1995; Tsujii et al., 1997; Fujita et al., 1998).

The current trial evaluated the COX-2 expression immunohistochemically in patients with GIST and investigated its association with mitosis, tumor size, stage, clinical behavior, surgical procedure and drug administration parameters.

Materials and Methods

Subjects

Forty-two patients (26 males, 16 females) diagnosed with GIST, who are under follow-up by the Medical Oncology and Pathology Departments at Gaziantep, Erciyes and Dokuz Eylul Universities were included in the trial. The file information of these patients was evaluated retrospectively.

Obtaining the Materials

The cases in this trial were detected by screening the record books and the computer achieves at the relevant Departments. The pathology reports generated by reviewing the materials (biopsies, organ resections) of intra-abdominal masses that are obtained for diagnostic purposes and sent to the Pathology Laboratory have been taken out from the archive. From the archive of preparations, histochemistry and immunohistochemistry stained preparations along with the hematoxylin & eosin-stained preparations of these cases with the same protocol were detected, and then appropriate preparations were selected, and paraffin blocks of these preparations were taken out from the archive.

Routine hematoxylin & eosin-stained preparations of

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all patients were re-investigated and the GIST diagnoses confirmed. CD-117, CD-34, S100, Actin, Vimentin, and Desmin, Ki67 were administered to all patients. PDGFRA was applied in a CD-117 negative patient and the diagnosis was established.

Non-stained Sections and Deparaffinization

Based on the investigation performed after appropriate block selection, an adequate number of non-stained sections were obtained. In this trial, Cox-2 (Santa Cruz, sc-7951) immunohistochemical marker was investigated. Lung adenocarcinoma sections were used as the control. Sections were made 5 micron thick by microtome and transferred to "polysine"-coated cover glasses to avoid spilling during staining. Cover glasses were kept at the incubator at 60°C for 30 minutes to enable adherence of tissues to the cover glasses. Cover glasses taken out of the incubator were kept in xylol for 10 minutes and subjected to deparaffinization.

Immunohistochemical Staining

Immunohistochemical staining was performed according to the following sequence:

1. Deparaffinized cover glasses were treated by xylol for 10 minutes, and then washed with 96%, 90%, 70% alcohol and water, respectively.
2. To increase the antigenicity of the tissues transferred to the cover glasses, concentrated "Citrate buffer 9003" solution was reconstituted by distilled water at a ratio of 1/10. These cover glasses prepared were placed in this solution and boiled in a microwave oven at 360°C twice in total at 9-minute periods. During these procedures, care was taken to keep the room temperature at 22°C.
3. The region surrounding the tissue was marked with an antibody pen so that the solutions applied are not dispersed.
4. The cover glasses are laid on the plastic containers, where the staining procedure will be performed, and kept for 5 minutes.
5. After keeping them in the super-block (Scytek REF:AAA125, LOT:11869) for 5 minutes, they were re-washed with PBS.
6. 5-minute application of 0.3% H₂O₂ was performed to avoid background staining.
7. Primary antibody was dropped, rewashing with PBS and kept for an hour.
8. After an hour, secondary antibody was dropped, washing with PBS (UltraTek Anti-Polyvalent Biotinylated Antibody, REF:ABN125, LOT:11881). After keeping in the secondary antibody for 10 minutes, washing with PBS was performed.
9. DAB was applied (Lab Vision REF:TA-012-HDC, LOT:HDC50706) and left for 5 minutes.
10. Washing with tap water was performed.
11. Washing with tap water was performed again after keeping in hematoxylin for a minute.
12. The cover glasses were covered after passing through alcohol series and xylol.

Scoring

Four to 6-micron sections prepared from the paraffin

blocks were stained by immunohistochemical method for COX-2 expression. Those stained at a low intensity, moderate intensity and high intensity were scored 1+, 2+ and 3+, respectively (Figure 1).

Statistical Methods

The statistical analysis and graphic drawings were performed using SPSS 11.0 and Microsoft Excell-2000 software. Descriptive statistics was used to summarize patient characteristics. Pearson's chi-square test was applied, using EpiInfo Version 6 for inter-group comparisons. $p < 0.05$ was considered significant.

Results

A total of 42 patients (26 males (61.9%) and 16 females (38.1%)) were included in the trial. All the clinical and pathological characteristics of patients were summarized in Table 1 by their COX-2 staining properties. The mean age was 56.85 ± 12.90 years (range: 32-77 years). Twenty-nine patients are alive (70.7%) while 12 (29.3%) died during follow-up. The mean follow-up duration was 34.96 ± 22.15 months (range: 3-120 months). In the evaluation of patients with respect to primary localization, the stomach, small intestine, large intestine and the other regions were detected to be the primary region in 50%,

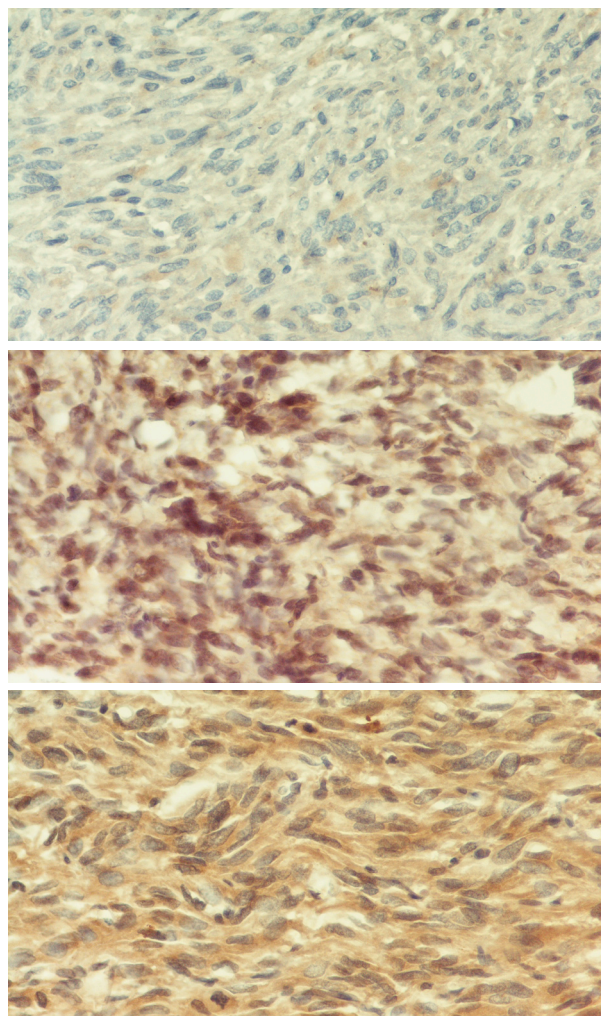


Figure 1. COX-2 Expression: a) Grade 1; b) Grade 2; c) Grade 3

Table 1. Clinicopathological Characteristics of Patients

| Variables | Subtype | Group A | Group B | p value |
|-------------------------------|-------------------|--------------|--------------|---------|
| Age (year) | | 57.37 ± 3.41 | 56.53 ± 2.48 | p >0.05 |
| Gender | Male | 10 | 16 | p >0.05 |
| | Female | 6 | 10 | |
| Duration of follow-up (month) | | 34.18 ± 3.92 | 35.26 ± 4.29 | p >0.05 |
| State | Alive | 11 | 17 | p >0.05 |
| | Dead | 3 | 9 | |
| Metastasis | Detected | 4 | 15 | p >0.05 |
| | Not detected | 10 | 11 | |
| Tumor size | <5 cm | 3 | 1 | p >0.05 |
| | 5-10 cm | 6 | 12 | |
| | >10 cm | 6 | 13 | |
| Mitosis | <5/BBA | 10 | 6 | p=0.02 |
| | 5-10/BBA | 2 | 7 | |
| | >10/BBA | 3 | 13 | |
| Risk Classification | Low risk | 3 | 0 | p >0.05 |
| | Moderate risk | 3 | 3 | |
| | High risk | 4 | 8 | |
| Medication | Not used | 8 | 12 | p >0.05 |
| | Imatinib mesylate | 5 | 9 | |
| | Sunitinib malate | 0 | 3 | |
| Operation | Resectable | 10 | 15 | p >0.05 |
| | Non-resectable | 4 | 11 | |

35.8%, 7.1% and 7.1% of the patients, respectively.

Patients were divided into two groups with respect to COX-2 expression; patients detected to have a staining intensity of 1+ or 2+ were classified as Group A and those with an intensity of 3+ were classified as Group B. There were 16 and 26 patients in Group A and B, respectively. The mean age of the first group was 57.37±3.41 while it was 56.53±2.48 in the second group. The mean duration of follow-up was 34.18±3.92 and 35.26±4.29 in Group A and Group B, respectively.

There was no significant difference between alive and dead patients with respect to COX-2 group. While mild to moderate COX-2 was detected in 11 of the 28 patients followed up as alive, 17 patients had intense staining. In 3 of the 12 dead cases, COX-2 was detected at mild to moderate intensity while 9 patients had intense staining (p=0.612).

There was no significant difference between patients with and without metastasis with respect to COX-2 group. While 4 and 15 of the 19 patients with metastasis had mild to moderate and intense staining, respectively, 10 and 11 of the 21 cases without metastasis had mild to moderate and intense staining, respectively (p=0.153).

Upon the investigation performed by dividing the patients into 3 groups by the pathologically determined tumor size (<5 cm, 5-10 cm, >10 cm), no significant inter-group difference was detected with respect to the COX-2 group. While mild to moderate COX-2 staining was detected in 3 of the 4 cases with a tumor size < 5 cm, only a single case was observed to have intense staining. 6 and 12 of the 18 cases with a tumor size at 5 to 10 cm had mild to moderate and intense staining, respectively. As for 19 cases with a tumor size > 10 cm, 6 and 13 patients had mild to moderate and intense staining, respectively (p=0.242).

Evaluation of all preparations with respect to mitosis (<5/50 BBA, 5-10/50 BBA, >10/50 BBA) detected a significant difference between the two groups (p=0.02).

inter-group evaluation achieved statistical significance.

After nonmetastatic patients were classified into 3 groups at GIST staging as those at mild risk, moderate risk and high risk, no significant difference was detected between the groups with respect to COX-2 group. In the current trial, no patient was detected, who was evaluated to be at very low risk. In all 3 patients in the low risk group, mild to moderate staining was observed. While mild to moderate staining was detected in 3 of the 6 patients in the moderate risk group, the other 3 had intense staining. 4 and 8 of the 12 patients at high risk were detected to have mild to moderate and intense staining, respectively (p=0.116).

In addition, the patients were divided into 3 groups as those undergoing complete mass resection who don't receive a tyrosine kinase inhibitor (imatinib mesylate), who receive imatinib mesylate and those receiving sunitinib malate after progression on imatinib; no significant difference was detected between these groups with respect to COX-2 group.

Among 20 patients undergoing complete mass resection, 8 had mild to moderate staining while 12 had intense staining. All three patients using sunitinib following progression on imatinib exhibited intense COX-2 staining (p=0.399). There was no significant difference between patients undergoing complete mass resection and those with no completely resectable mass with respect to COX-2 group. While 10 of 25 patients undergoing complete mass resection at operation had mild to moderate staining, 15 had intense staining. 4 and 11 of the 15 patients with no completely resectable mass had mild to moderate and intense staining, respectively (p=0.607).

Discussion

In many cancers, the association of increased expression of COX-2 and disease progression, and the progression-delaying and/or therapeutical effects of

COX-2 inhibitors are established. The relation of COX-2 expression and GIST is a very new investigational subject, and there are limited trials on this subject (Sheehan et al., 2003, Stewart et al., 2006, Stepanova et al., 2005; Gumurdulu et al., 2007). There are many trials on prognosis and survival analysis investigating COX-2 expression in various cancer types, and primarily colon cancer; however, conduct of trials on prognosis and survival analysis in GIST is difficult since such tumors are relatively rare.

The introduction of imatinib mesylate in the last 5 years has blazed a trail in GIST treatment. Although wide-scale analyses are not feasible, imatinib markedly increases the survival in clinically recurrent or metastatic GIST cases (Demetri et al., 2002; Siberman et al., 2002). The first trial on COX-2 expression in GIST was reported by Sheehan et al, in which 80% of the cases exhibited COX-2 positiveness (Sheehan et al., 2003). We believe that inclusion of only 15 cases is a weak aspect of the trial. COX-2 expression was observed in both epithelial- and the spindle-cell subtypes of tumor samples. Based on the data obtained, authors reported that COX-2 inhibitors could be beneficial in GIST although these tumors are not responsive to chemotherapy and radiotherapy, and partial response to tyrosine kinase inhibitors is obtained. In the trial by Stewart et al, a higher tumor recurrence and/or death was observed in the group with a low or limited COX-2 expression, as well as the COX-2 positiveness in 92% of the cases; however no statistical difference was detected (Stewart et al., 2006).

In the report presented by Stepanova et al at 2005 American Clinical Oncology Association, COX-2 positiveness was observed only at a rate of 27% and COX-2 was suggested not to provide a significant difference with respect to time to progression (Stepanova et al., 2005). However this trial has not been published yet as a complete article.

There are few trials determining the relation between COX-2 and GIST tumors. The association between COX-2 staining and survival could not be detected. The reason is the inadequate number of patient deaths following imatinib treatment as the primary endpoint for such an analysis. Longer follow-up is required for such analysis.

In the current trial, the association between the mitosis rates in tumor cells and the tumor size was found to be significant. In fact, this is an expected result. Similar results were also reported in the previous trials. However, the current trial detected a significant correlation between the mitosis number and the COX-2 expression ($p=0.02$), differently from the trial by Stewart et al ($p=0.02$) (Sevinc A, 2007). However, there was no significant correlation between the COX-2 expression and the tumor size ($p=0.242$). In contrast to the current trial's result, there was a negative correlation between the mitosis number and the COX-2 expression in the trial by Stewart et al (Stewart et al., 2006). In another trial by Gumurdulu et al., no correlation was reported between mitosis and COX-2 expression (Gumurdulu et al., 2007). To achieve a definite interpretation on this subject, trials on survival analysis are required to be continued, thereby demonstrating the correlation between COX-2 and

prognosis. The statistically significant correlation between COX-2 expression and the mitosis number detected in the current trial strengthens the possibility of COX-2 to be an independent risk factor. It would not be inaccurate to state that COX-2 expression is higher in tumors with a large number of mitosis. Regression analyses including a larger number of patients are required to comment on which is secondary to the other. In brief, COX-2 expression may be correlated with prognosis; if this can be confirmed, the use of COX-2 inhibitors as supportive therapy may be introduced. The available data don't allow us to make a reliable comment on survival between tumors with a high and low COX-2 expression.

After nonmetastatic patients were classified into 3 groups at GIST staging as those at mild risk, moderate risk and high risk, no significant difference was detected between the groups with respect to COX-2 group. In addition, the main important result in the current trial was the association between the disease stage and COX-2 expression. Although the mitotic index is high in certain tumors, this doesn't correlate with the tumor size. This may be explained by the early manifestation of tumors depending on their localization. It's quite difficult to find an objective evidence of such an explanation. Therefore, 2 prognostic criteria should be considered when performing a prognostic assessment in nonmetastatic GISTs. These include the number of mitosis and the tumor size. The risk staging of tumors is performed by using these two parameters. Risk classification is possible by either of the two parameters. Tumor size (>10 cm) and the number of mitosis (>10 /HMF) are criteria indicating poor prognosis, together or separately, which demonstrate the high risk in patients. The correlation between the increase in mitosis number and COX-2 expression detected in the current trial suggests that COX-2 expression could be used as a prognostic criterion. While no correlation was detected between the COX-2 expression and tumor size in the current trial ($p=0.116$), there was a significant correlation with the mitosis number ($p=0.02$). The investigation of 16 cases with a mitosis number >16 revealed 3 and 13 cases of intense staining in Group A and B, respectively. Similarly, while Stewart et al detected no correlation between the tumor size and COX-2, they reported an inverse correlation with mitosis number in contrast to the results of the current trial (Stewart et al., 2006).

Based on currently available data, there is no clear explanation for the use of imatinib mesylate by high-risk patients, who have undergone complete resection; trials on this subject will be completed in 2008. Multi-center trials involving a large patient participation may demonstrate the determinative role of COX-2 expression in this risk staging

No significant correlation was detected between imatinib use and COX-2 expression in the current trial, except these data ($p=0.399$). 5 and 9 of the 16 patients using imatinib had mild to moderate and intense staining, respectively. All three patients using sunitinib following progression on imatinib exhibited intense COX-2 staining; however no statistical inter-group difference was detected due to the inadequate number of patients. Remarkably, there was a relative correlation between the increase in COX-2 staining intensity and imatinib use. An intense

staining pattern was observed in patients with a poor prognosis and 3 imatinib-resistant cases. Based on this, the correlation of COX-2 expression with prognosis may be indirectly suggested.

In addition, the mean duration of survival of patients undergoing total resection and not receiving imatinib could not be determined because prognosis is already good in patients receiving surgery as the primary treatment. As for relapsed patients or those who were metastatic at study baseline, prognosis markedly improved in our series compared to pre-imatinib period. Among the group of patients on imatinib treatment, who are monitored at Gaziantep University Medical Oncology Clinic (n=20) for the last 5 years, only 3 patients died and the other patients are still under follow-up on treatment. The marked improvement in duration of survival detected in these patients demonstrates the efficacy of imatinib treatment. In this period of time, 3 patients were detected to have imatinib resistance and were shifted to sunitinib malate treatment. Application was submitted to and approval was obtained from the international support program to start nilotinib, a new tyrosine kinase inhibitor, in a patient developing resistance to sunitinib.

Another result we would like to indicate is that CD-117 was positive in 98% of our population. Imatinib treatment was initiated in a CD-117-negative patient upon demonstration of positiveness by PDGFR staining, and significant improvement was achieved in clinical parameters by treatment (Sevinc et al., 2007). Therefore, a treatment regimen guided by only CD-117 positiveness may be inaccurate in GIST cases. Clinical experience and prognosis should be evaluated together with laboratory tests.

GIST is an extraordinary and interesting disease both to physicians and patients. Medical oncologists are divided into two groups according to the cancer type they are treating: Oncologists and OncoGISTs. Every patient with GIST is a new experience for a medical oncoGIST (Sevinc et al., 2008; 2009).

Acknowledgements

All authors participated in the critical review of this report for its intellectual content. AS and CC had the original idea and wrote the first draft of the paper. In particular, IS, IS, and OS performed the pathological analysis. AS, MEK, OE, MD, UY, and AA contributed patient data and samples. All authors participated in the undertaking, data analysis, and data interpretation and participated in drafting, revising, and finalising this report. All authors participated in developing the study concept, laboratory analysis, data interpretation, and preparation of the report for publication. The authors declared no conflicts of interest.

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